

nature*December 4, 1975*

21st century food needs thought — now

“OUR understanding of taste physiology and of flavour technology is abysmal. If the next generation and generations beyond are going to be fed acceptably as well as adequately, we shall certainly need to do far more research and development in these fields of study than has been contemplated hitherto . . . in my opinion the survival of our western civilisation is going to depend on a new food revolution—by which I mean a revolution in our methods of processing food, in increasing the shelf-life of our food, and in improving our methods of distribution. In practically all advanced countries agricultural research still attracts vastly greater resources than does food research. This imbalance will surely have to change.”

Those words of Lord Zuckerman at the British Nutrition Foundation's Annual Lunch brought loud ‘hear-hears’, banging on tables and tapping of coffee-cups. They obviously struck a responsive chord amongst the mixed audience of food industrialists, academics and civil servants, but that is hardly a surprise since more research and development means more jobs and more government money. Is there, however, more to this than the conventional response of any interest group?

The more one looks at the sciences and technologies connected with food, the more one realises how ignorant we are and how unscientific our approach is to food. This is, strangely, not a statement that has to be dragged out of a profession in the face of massive public relations effort to convince one that all is well and that there are many fine minds at work; it is one that is heard regularly and almost despairingly from the practitioners themselves. It seems that it is often almost impossible to attract bright students into the field; mainstream academic thinking (with the encouragement of the Nobel committee) must take the blame for much of that. It is praiseworthy to have developed a new and better strain of rice, or to have worked out at the molecular level how something works. But to have developed a new means of preserving food, or a way to persuade the average citizen to consume textured vegetable protein; these are mere technical advances which can presumably be done by people with lesser qualifications.

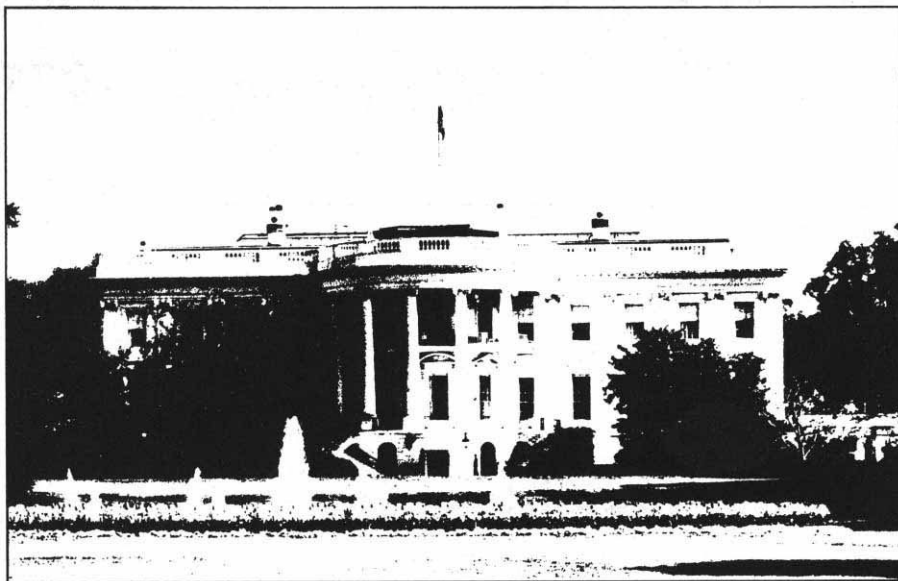
Another disincentive to the bright youngster is the evidence, all around us, that we are better fed than ever before. In Britain there is plenty of variety—the nutritionist's first requirement—and the calorie value of food ‘moving into consumption’ is 10 % higher per capita than it was in 1945 and a full third higher than the national average requirement, permitting us to be more wasteful. Nutritional diseases are rarer (except for dental caries)—surely everything challenging has been done already.

The same sort of things were being said in the 1960s about energy, and now we are busy looking for alternative sources and ways to persuade the public to be energy-conscious. Food has already sounded one early warning of future crisis; surely now is the time to be doing intensive research on food options including acceptability, for the 1980s and beyond. There is no sign that anything like enough is being done.

To pursue the analogy with energy, one of the weaknesses of the present “save it” campaign is that it is trying to put into reverse the “use it” advertising so common until recently alongside which the government took no discernible action. The lesson for food must be that whilst there is still great variety and whilst quantity is no problem, there must be a growing governmental involvement in providing information and education. If launched now, such a campaign need have no negative overtones but could instead encourage the great eating public to be participators in a genuine experiment to evolve new, acceptable foods. Adult tastes can change (or rather, grow)—the recent proliferation of take-away Indian and Chinese restaurants is testimony to that—but the increase of nutritional awareness amongst children is probably a higher priority. The daily meal at school provides an excellent focal point for the discussion of nutritional matters; already a few schools permit their pupils to help plan weekly menus. This sort of practical involvement is an ideal form of education.

We have only two options available in thinking about future food supplies. The first is to hope that the crunch will not come in our lifetime. The second is to invest heavily in research, development and education so that if the crunch does come we can confront it in a prepared and mature way. □





Wanted : new science policy

In the United States, as in the rest of the developed world, the twin-headed international monster of simultaneous inflation and recession has, by threatening an era of no growth, loosened the financial adhesive that has until now made technology and progress virtually synonymous. It may be time for innovation itself to take a new form. Wil Lepkowski reports.

FOUR years ago, as President Nixon was preparing schemes for waging his re-election, one of his aides took the notion that "technology" might be a viable campaign tool for harnessing what was left of a sympathetic technical community. Out of the woodwork flew William M. Magruder (no relation to Watergate's Jeb S. Magruder), who until then had been the government's supersonic transport lobbyist. History records that Magruder, aeronautical engineer and former test pilot, failed in his attempt to sell Congress on the merits of supersonic passenger flight. But Nixon gave him a second chance. He appointed him to assemble a "New Technological Opportunities Programme".

That failed, too. Nixon's own Office of Management and Budget allowed Magruder to huff and puff in a vast interagency search for the finest, economically most relevant set of programmes. But it saw that the best ideas were already being funded and the "new opportunities" were simply unfunded dreams taken from dusty file cabinets. Nixon subsequently unfurled a platitudinous science and technology message in March 1972 (less than a year before he wiped out the White House science advisory apparatus). Magruder was given a large office to

rethink his goals, and the myth that crewcut, phallic technology was the answer to America's challenge of the 1970s sputtered to an end.

The economics of America weren't good then, and heaven knows they are much worse now. Yet curiously, Nixon's successor, Gerald Ford, wheels out no latter day Magruder. William M.'s perceptions, like Jeb S.'s ethical compass, were off by something like 135° in a value system that continued to stress large scale, power hungry, complex systems at the expense of the sophisticated but smaller scale technologies that seem needed today. The curiosity is that Ford, who, in a man by the name of William Goldwin has an intellectual advisor to flag him on critical future issues, hasn't uttered the slightest hint that maybe a new look at technological innovation and the scientific base needed to sustain it might be part of the way to solve the country's potentially disastrous outlook.

Technical Washington is tired, dejected, and cynical—a virtual burnt-out case. "There seems to be a malaise that has set in," says a National Academy of Sciences staff member, "and it has deepened. Members of the committees I work with seem to feel that nothing they do matters".

Big technology, however, does churn

forward, chiefly in the form of energy projects under the Energy Research and Development Administration. And in the field of health services, technological impersonalisation in the name of productivity holds policy sway, in spite of the Department of Health, Education, and Welfare's new dedication to prevention as the programme perspective for the next 10 years. In both fields, central to national welfare, costs and consumer dissatisfaction soar. One Washington source observes that present leaders continue to wear the same perceptual filters that brought the country to its present burnt-out state. He says we need a new set.

The new set could come in the form of a thoroughly new look at innovation for an era of no growth. Merely beginning the exercise would be monumental in itself, for the assumption is that economic growth must resume if any innovation can happen. More to the economic point, conventional wisdom has it that the economy simply must grow if American industry is to raise the capital to build the machines to provide the jobs to prevent social unrest. Industry needs around \$4.5 million million in new capital by 1985 to do little more than stay even. Few of the best banking heads know where even half that will come from.

There is trouble ahead, and if there was ever a time for the generation of new ideas it is now. The maddening fact, however, is the almost complete lack of debate over technological and scientific innovation. Almost . . . for there is a twinkle.

The twinkle is Princeton University economist Robert Gilpin, who has produced a report for the economic growth subcommittee of the joint economic committee. Gilpin's report lacks the substance that could come from discussing 'things' in the form of the ideas and inventions that offer a way out of the mess. But he gives broad policy hints that say we should at least be nimble.

“The maddening fact is the almost complete lack of debate over technological and scientific innovation”

“The manner in which we manage the so-called energy crisis will be very instructive with respect to our capacity to rejuvenate our economy in an intelligent way,” he says. “On the one hand, the resource, environmental, and related problems affecting our economy may very well be the functional equivalent of catastrophe. They can and could force the long-term rebuilding of our technological-industrial infrastructure. New demands and needs have

been created which, with proper incentives, could lead to the innovation of new industries and technologies which in turn will generate new technology-intensive exports.

"On the other hand, in our anxiety to find a quick and short term solution to our energy and related problems we could, through government subsidisation of large and commercially inefficient technologies, harm our economy. In pursuit of energy independence and security of supply, we may lock our economy into a high, non-competitive price of energy." He might also have added health and welfare costs.

Gilpin calls for more planning of America's future. But his caution is that economic planning in the past has been justified usually in terms of protecting rather than rejuvenating industry. What seems to be needed is

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a thorough examination of the scientific enterprise and the ways its own mode of operation is governed by out-moded economic and industrial priorities.

The point is that the issue of innovation during a time of sweeping economic change—and the anxiety that accompanies it—is crucial and necessary, but tricky. It calls for breaking through to new perceptions without falling into the traps of faddism and technological cynicism.

All over the country, small groups of scientists, technologists, artists, children and plain people are forming communities built on small scale self-sufficiency technology. To them, this is innovation. And having given up on their government, they are going their own way with the old string and sealing wax approach. Except they understand optics and electronics and mechanical inventions as well. Strangely, neither Ford nor the Energy Research and Development Administration, nor the Department of Housing and Urban Development, nor the Office of Technology Assessment in Congress are doing anything to tap this potential rescue army.

Meanwhile, its troops buzz away constructing their beehives according to the laws governing the economics of nature. Others are designing new health maintenance systems built on the premise that the body tends to heal itself and can be promoted to do so without heavy resort to medications. And no one up high notices them for the innovations they could represent. The

worst offender is none other than the National Science Foundation, whose mission is to search out and fertilise the innovator.

All of this is understandable, however. The 'new innovators' aren't always the most patient thinkers, and many are outright anarchists. One is Karl Hess, Barry Goldwater's former speechwriter, who in one motion evolved from conservative to radical in his politics, now refuses to pay any income tax—claiming to own nothing—and is working on a neighbourhood self-sufficiency project in a Washington ghetto. Still, Hess and hundreds of other such community leaders may have something if neighbourhoods in the middle class suddenly find their natural gas supply at an end and jobs not available.

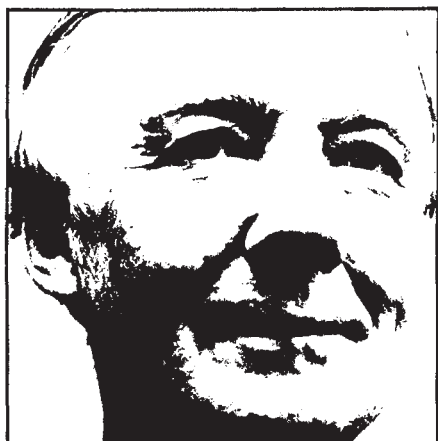
Perhaps one of the problems is that Ford and his advisers refuse to consider the worst of all possible futures (such as the collapse of the central banks) when technology for self-sufficiency would be the policy.

A policy for the worst possible future would create the lean and elegant technologies that just could turn the society back to a sense of benignity and small scale. There are some harsh economics truths in the air at present that indicate the advisability of a 'new science policy'. The financial markets are in virtual turmoil, owing to the barely averted collapse of New York City and, with it, New York State. The ripple effect through the entire socio-economic structure of the country is too vast to contemplate if the country cannot pay its bills to itself. Everything stops, except the 'life urge' itself—and that is the ultimate of any economic or science policy; human needs. There is no fooling around about such matters. But, curiously, leaders are ignoring them. □



Ford: should he consider a technology for self-sufficiency?

Behind any cod peace



KENNETH MELLANBY

FISH, as all admirers of the late P. G. Wodehouse's Bertie Wooster well know, is a food which nourishes the brain. Before he tackled any particularly taxing problem, Jeeves was urged to consume a dish of cod. It is to be hoped that all those trying to solve the problems of fishing in Iceland's territorial waters will keep their mental faculties in tip-top order by themselves eating some of the fish which are the cause of the trouble!

The problem is one of marine conservation. Catches of cod and other fish off the coast of Iceland have been falling and biologists believe that unless the tonnage caught is substantially reduced, the industry will suffer permanent damage. This would be a major disaster to Iceland. As far as Britain is concerned, the livelihood of a proportion of our 17,000 fishermen, and of the east coast ports from which they operate, would be affected, but our national economy is hardly at stake. Nevertheless we would clearly benefit if we could continue to share in a sustainable yield, and we could make long term plans accordingly.

The importance of fish in our diet has often been overestimated. When there was a general belief in a global "protein gap" there was a hope that the oceans would make a substantial contribution to offset shortages of essential elements from crops and animals grown on dry land. The high levels of first class proteins in fish made them particularly valuable in this connection. A great deal of research has been done to try to maintain and increase fish stocks, and to develop fish farming in both marine and freshwater habitats.

Nevertheless, in most western countries, fish consumption has steadily decreased. During the nineteen thirties people in Britain ate on an average

some 30 pounds of fish annually, and it is reckoned that over 70 per cent of white fish went to fish and chip shops to provide a cheap and nutritious meal for the poorer sections of the population. Today we only eat about 12 pounds a year—some four ounces a week. Fish and chip shops have disappeared from many towns, or deal mainly in other foods. Many towns no longer support a "real" wet fish shop. The main source of fish for most of our population is the frozen food cabinet in the supermarket and the most popular dish the fish finger (which, incidentally, may have a higher content of batter and other extraneous matter than of actual fish).

The situation that now exists is that, except in a few communities in maritime areas, fish is of little dietary importance. It does indeed contain first class protein, but few people would be deficient without it. The contribution to the calorific intake even in those areas where most is eaten is almost negligible, for weight for weight cod has a lower energy value than almost any other widely consumed food. From the point of view of diet the vitamin D in the fish liver may be important, for a shortage of this substance appears to be increasing particularly in children of Asiatic origin coming from sunnier countries where the vitamin was synthesised in their skin. Each year the medical literature reports an increasing number of cases of rickets.

Sea fish and its waste also contributes to pet foods, to animal feeding stuffs and to fish manure. But the largest amounts of fish products used

in this way include such substances as the Peruvian anchovy, rather than the catches of our British-based fleet.

Notwithstanding the comparative unimportance of fish to our nutrition, the industry is an important one, and this should be the basis of negotiations with the Icelandic government, and of international discussions shortly to take place on the Law of the Sea and of the rights of maritime and fishing nations in the water off their coasts. It is to be hoped that "red herrings" such as the protein levels in the diets of Western nations will not be allowed to obscure the issues. Eating fish gives pleasure to many, and makes their diet less monotonous. Our fishermen have hard and dangerous lives, and deserve to be adequately rewarded. Research has taught us a lot about population dynamics and about how fish stocks can be conserved, though some of the recent changes in numbers and distribution—for instance the disappearance of herring from many traditional fishing grounds—are not fully understood.

It looks as if considerable restraint in fishing will be needed in breeding areas such as that around Iceland, and that increased catches of species which the housewife presently finds unacceptable may be needed if consumption is not to fall even further. Fish farming in sea lochs and freshwater will no doubt increase, but it is unlikely that fish will ever form more than a tiny fraction of the world's food supply. The growing world population will need to be fed on the increased productivity of terrestrial rather than of marine crops and animals.

As for the worry about the energy used in modern farming, where the crop produces fewer calories than are expended in producing it, this applies even more to fisheries. The fishermen themselves consume about a quarter of the calories of their catch, their boats use many times as much energy, and this total is further increased when that needed to build the ships and manufacture the fishing gear is included.

● Mr Jens Evensen, Norway's minister for matters relating to the law of the sea, has suggested a law empowering the Norwegian Government to introduce a 200-mile economic zone off the country's coasts. The suggestion, which came in a report being debated this week by the Norwegian parliament, is designed to prepare in advance the legislation that would be necessary when a solution is found to the problem of extending Norway's fishing limits. It allows for the introduction of the wider non-trawling zone gradually, and also for narrower limits that relate to types of catches and gear at different times of the year. □



Cartoon: Mahood, Financial Times

"For us it could be
'Peace in Our Time' "

international news

Appointment to Soviet Academy threatens autonomy

from Vera Rich

THE election of Academician Anatolii Petrovich Aleksandrov as President of the Soviet Academy of Sciences, to replace the interim President appointed last May, Academician Vladimir A. Kotelnikov, took place in an atmosphere which may well indicate a greater degree of governmental supervision of the Academy's work in the future.

It is significant that in the election of the last two presidents, Nesmeyanov in 1951 and Keldysh in 1961, it was an academician who proposed their candidature to the general meeting of the Academy. For on this occasion, following a brief statement from Kotelnikov that the Presidium of the Academy had recommended Aleksandrov for the post of President, the main speech of proposal was made, not by a member of the Academy, but by the Secretary of the Central Committee of the Communist Party, M. A. Suslov. Furthermore, in addition to proposing Aleksandrov, who is also a member of the Central Committee, Suslov took the opportunity to outline what may well prove to be the official science policy of the Party for the next five years.

While stressing that "the Party gives foremost significance to fundamental research", he also stressed the importance of fundamental research for "all aspects of human activity, increasing the efficiency of social production, the social consequences of the scientific and technological revolution" and laying "new main lines of scientific and technical progress." Quoting Brezhnev's speech at the recent Jubilee celebrations of the Academy, he reiterated that "the Party expects from scientists ever deeper and bolder investigations into new processes and phenomena, an active contribution to the work of scientific and technical progress, a thoughtful analysis of problems which arise, and responsible recommendations on the best means of solving them in the interests of strengthening the power of the country, improving the life of the people, in the interest of the building of Communism".

Drawing attention to the forthcoming 25th Conference of the Party, which will approve the "Theses" of the next five year plan, Suslov said that this would be a plan of "quality and productivity", in which science would be expected to play its part in applying the results of basic research to technological practice and emphasis would be put on a closer connection between research and material production. It would therefore be necessary, he asserted, to improve the control of all academic establishments throughout the Soviet Union, so as to establish a "united system, working clearly and in coordination". This system would include the Academy itself, together with its various new scientific centres and the Academies of the Union Republics. Attention must be given, he added, to those Institutes of the Academy where there are still "insufficiencies and unresolved problems", in order to utilize fully the "enormous scientific potential" of the country. A greater return is thus expected on government investment in a science.

This is, of course, a politician's speech, and perhaps would merit less attention had it been delivered simply as an address from the Party Secretary to the meeting of the Academy. However, by making such a statement in a speech proposing the new President of the Academy, it might also be construed as presaging a greater involvement of the Party in the running of the Academy. It is noteworthy in this respect that, while Keldysh was elected to the Central Committee of the CPSU in 1961, the same year as he took office as President of the Academy, Aleksandrov has already been a member of the Central Committee for nine years, and his involvement in the higher echelons of Party life must accordingly be considerably greater.

Aleksandrov is, of course, well-fitted scientifically for the prestigious post of President of the Academy. His scientific interests are wide ranging, and range from high-polymer compounds to nuclear energy. In the 1930s he was part of Ioffe's nuclear research team in Leningrad, and later worked with Kurchatov, whom he succeeded in 1960 as director of the Kurchatov Physics Institute of the Academy. He has won both the Lenin and Stalin (now State) Prizes for science and holds a number of Soviet awards in-

cluding the Order of Lenin, which he has received seven times. He is well-known internationally for his participation in the Geneva Conference on the peaceful uses of atomic energy in 1958 and the sixth Pugwash Conference. He also has considerable experience in practical applications of fundamental research and in the coordination of research activities, and is a skilled scientific administrator. In effect, on the basis of his curriculum vitae, and taking into account the trend of Party policy for science as outlined in Suslov's speech, he would seem an ideal candidate.

There is, however, one major factor against him. At 72 years of age he is older than both Kotelnikov and Keldysh. He can hardly hope for a long term of office, and, it would seem, must be regarded as effectively another interim President. The greatest significance of his election for the future of the Academy may well be the manner of it. The Academy has always attempted to maintain an autonomous status in Soviet life. The close participation of Party Secretary Suslov in the election of the new President may well presage a situation in which its independence will in the future be considerably more circumscribed. □

Nuclear exporters agree safeguards

FOLLOWING a series of secret meetings held in London since last April, senior officials representing the seven industrial nations which supply most of the world's exports of nuclear materials have reportedly reached agreement on means of preventing the adaptation of nuclear technology to military ends by other countries.

The Nuclear Suppliers Group, or Group of Seven, came together following India's explosion of a nuclear device based on imported Canadian technology. It consists of the UK, the USA, the USSR, France, West Germany, Canada and Japan. The notable exception—China—is thought not to be ready to export nuclear technology at the present time.

The agreement seeks to establish more stringent safeguards governing the ways in which nuclear material, equipment and expertise is used by the Group's customers. It does not

apparently cover the export of nuclear reactors, focusing rather on uranium enrichment, the reprocessing of nuclear fuel and the production of heavy water. The precise details remain a secret, however, though the nuclear countries may want explicit undertakings from their customers that they will not use nuclear material supplied for civil purposes to produce nuclear weapons.

It is understood that each party to the agreement will simply inform the others of its intentions to observe the agreed safeguards, which are expected to be presented at the meeting in Vienna early next year of the Board of Governors of the International Atomic Energy Agency (IAEA).

There may also be a proposal at the meeting to make more countries subscribe to the IAEA's existing safeguards. At present they apply only to countries which are signatories to the nuclear Non-proliferation Treaty, but the hope is that countries like Brazil, Egypt, Israel, India and Pakistan—all of which have not signed the treaty—might state their intentions more specifically if they are given the clear option of agreeing to the safeguards.

It is also hoped that other countries with the potential to become exporters, like Sweden, Holland and South Africa, will adopt the new code just agreed by the Group of Seven. But whatever the outcome, a central problem remains: it is not certain that a particular nuclear experiment—such as an underground explosion—can be established, to the satisfaction of the group or anyone else, as essential to the development of nuclear energy, rather than a preliminary step to the manufacture of a nuclear weapon. Which, given India's case, is where the Nuclear Suppliers Group came in. □

Moves to keep the dragon

It now seems that the UK-based OECD Dragon High Temperature Reactor Project, the continued existence of which has been in doubt for the past few months, will carry on for at least nine months. Compromise proposals made last week in Brussels, but subject to clarification in further meetings this week, mean that there will be time for the Dragon Board of Management to sort out the bones of an agreement with the US Energy Research and Development Administration (ERDA), which has expressed interest in using the Dragon reactor at Winfrith, Dorset.

The villain of the piece is widely acknowledged to be the UK. The De-

partment of Energy, presumably egged on by the Treasury, has in the recent past expressed complete reluctance to the idea of contributing any more money to a project which it now sees as well outside the mainstream of British reactor development—at present centred on the 'Steamer' pressure-tube reactor, and, in the longer term, the Fast Breeder Reactor.

Back in September, formal steps had to be taken to run the project down because there seemed no prospect of agreement about future funding. Then the UK relented a little by agreeing to a stay of execution until the end of March next year. Now West Germany has apparently convinced the EEC to see that deadline extended by a further six months.

The EEC is closely involved because Euratom is one of the participants in the project; smaller participating countries like Austria, Switzerland and Sweden have said that they are happy to go along with the German proposal, so Brussels calls the tune. What remains to be clarified this week is the precise meaning of a UK suggestion to the effect that satisfactory financial arrangements must be on the horizon by the end of March.

Dr L. R. Shepherd, chief executive of the Dragon project, said last week that he hoped the UK would adopt a reasonable approach to the problems, and not stick too rigidly to its argument that Dragon is no longer of any direct use to the UK's nuclear programme. Whatever mistakes the UK may have made in its Advanced Gas-cooled Reactor programme, he said, it still has unsurpassed experience of gas-cooled reactors, and it would be folly to opt out at a time when the ERDA is looking favourably on collaboration.

Quite apart from that, the United Kingdom Atomic Energy Authority does quite nicely out of Dragon because, even now, it gets back from the project more money in payment for services and so on at its Winfrith site than the UK pays out to be a member of the Dragon project. If ERDA makes a contribution, and the UK pays less as a consequence, the situation will look even more attractive.

That said, however, it needs to be remembered that ERDA is rather unsure of its position at the moment because General Atomic in the United States has been busily losing all its commercial orders for high temperature reactors during the past few months—partly because electricity utilities in the USA are short of money and partly because the anti-nuclear lobby has been so conspicuously successful in getting its message across. □

Indians ask for more nuclear power

Jullundur

INDIA has yet to display the trends apparent in small-town communities in the United States which react strongly to moves to establish nuclear power plants in their areas out of concern over possible radiation hazards. On the sub-continent nuclear energy continues to rate highly. Most people consider it prestigious and modern.

With the number of reactors in India still relatively small, most people are both unaware and unconcerned about the hazardous aspects of nuclear energy. In fact, one of the most persistent gripes that eastern states have had is that the Atomic Energy Commission (AEC) has time and again refused to concede their 'just' demand for a nuclear power station in their regions. The state of West Bengal has actually made it a cause, leading a vigorous battle on the issue.

The AEC's long-held view is that nuclear power generation in the eastern region, where most of the country's coal mines are located, is neither necessary nor economically viable. An AEC study concluded that nuclear power within 800 km of a coal pit-head would be uneconomical, and two further detailed studies carried out by the Central Planning Commission suggested that there was no need for a nuclear power station in the eastern region.

Not to be deterred, the West Bengal government pressed its demand. Then came the oil crisis, which pushed up all prices, including that of coal. Freight rates also went up, making coal haulage more expensive, and the West Bengal government pressed its claim for nuclear power even more vigorously.

The central government may finally have to yield to regional pressure. An official of the West Bengal government has claimed that studies carried out by the AEC in 1973-74 had reduced the break-even radius for nuclear power to only 300 km, with the "changed economics of coal", and the new studies would further reduce the radius to less than 150 km.

If this happens, it would help "justify" the West Bengal demand for a nuclear power station, because the site the state had proposed for the purpose is situated some 250 km from the Asansol coal mines. Although West Bengal is an industrially advanced state by comparison with others which already have nuclear power, it remains debatable whether there is much to justify its continued insistence on having a nuclear power station—whatever the economics of coal. □

WHEN Myron B. Kratzer, Acting Assistant Secretary of State for Oceans and International Environmental and Scientific Affairs in the USA, spoke about international cooperation in nuclear energy in Stockholm recently, last week's agreement in London between the world's seven major nuclear suppliers had not been concluded. That was probably just as well. His speech was unrealistically optimistic as it was, without also voicing the official claims that the London agreement will probably precipitate: that the risk of the proliferation of nuclear weapons has been minimised.

Mr Kratzer was addressing the Atomic Industrial Forum on the Nuclear Fuel Cycle. His assessment that the International Atomic Energy Agency safeguards and the Nuclear Non-proliferation Treaty (NPT) have served their purpose well in stopping the proliferation of nuclear weapons can hardly be taken seriously in view of the disastrous results of last May's NPT review conference. Those signatories who attended were unable to agree on any of the major measures proposed to help stop proliferation. In general, the strength of the NPT has been sapped by the failure of the nuclear-weapon parties to the Treaty to fulfil the few obligations it demands of them. And, of course, France and China—as well as many near-nuclear states—have not even signed it.

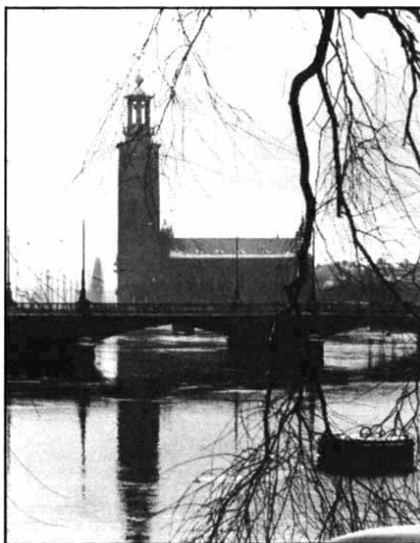
To maintain, with Mr Kratzer, that “the record of the past 20 years on international nuclear cooperation gives considerable basis for optimism that nuclear development . . . can take place without contributing to further proliferation” is to ignore the factors which will make the next 20 years different from the last: a level of technical sophistication which will enable many states to manufacture their own nuclear weapons, the really dramatic spread of nuclear technology, and the conviction of the non-nuclear states—fostered by the behaviour of those states possessing nuclear weapons—that such weapons are desirable for national security.

● Sweden's new International Energy Institute, scheduled to open at the beginning of 1977, might do well to consider some of the areas recommended for research by a recent gathering of international energy-oriented academics near the southern city of Gothenburg. The meeting was arranged by the Royal Swedish Academy of Science in conjunction with the Natural Sciences Research Council and the Atomic Research Council.

The research suggestions concerning energy in less developed countries sounded the more convincing for the inclusion in the group of the Sudanese Minister of Agriculture, Dr Hussein Idris. Pointing out that an injection of energy into an almost feudal society can widen the gap between rich and poor, the group urged

Letter from Sweden

from Wendy Barnaby, Stockholm



that research take into account the social as well as the biological aspects of ways of solving the immediate problems: the production of food and raw materials.

Other proposals to emerge from the meeting suggested a look at alternative ways of using energy, especially in industry. The academics called for the development of a clean method of burning coal, and advocated a study of the problems of the breeder reactor.

The group studying the side effects of energy production and use suggested that cost-risk-benefit analyses of different energy sources be undertaken, and urged that the side effects of each source be identified and used as resources in some other activity. This group produced a very wide-ranging and valuable list of research areas, including the effect of energy production and use on the Earth's climate, the ways in which scarcity of energy could lead to conflict and the effect of fast-changing technology on social relations. Also proposed were studies of the biologically disturbing effects of different energy systems on different levels of organisation (cell, organism, experimental animals, ecological systems) to determine the mechanisms whereby energy systems cause damage to health; analyses of

the effects of energy use on man's health, using information from registers of cancers and birth defects; an examination of energy wastage; and an assessment of the harmful effects of energy production and use in nature.

● Changes in the climate of northern Europe have been blamed for the decrease in the number of eels in Swedish waters. Professor Gunnar Svärdson, working at a freshwater laboratory near Stockholm, has concluded that the usually cited local villains—pollution of the Baltic and overfishing—are not to blame.

Between 1964 and 1969, the average annual catch of eels in Sweden was 1,380 tons. Between 1969 and 1973, this figure dropped to 955 tons. The eels come to Scandinavia through the North Sea from the Atlantic, and on their arrival in Sweden are counted at collection stations and distributed throughout various rivers. As the 20 stations whose records are available all agree that the numbers arriving have decreased, Professor Svärdson turned his attention to the factors stopping their arrival, rather than the conditions they find when they reach Swedish waters.

One such factor is the temperature of the North Sea, which, with the exception of the past three years, has been falling steadily over the past three-and-a-half decades. Although the drop is not measurable in terms of degrees, it has nevertheless significantly decreased the amount of plant and animal plankton, an important food for eel larvae and young eels. At the same time, Arctic fish such as cod have increased their visits to the North Sea—and cod love to eat eel larvae. Meeting less food and more enemies, fewer eels survive their passage across the North Sea.

Like the water temperature, the pattern of prevailing winds has also altered. The westerly winds which sweep over the British Isles and help to point the eels in the direction of Scandinavia blow on only 80 days a year now, instead of 110 as used to be the case. Most of the newly calm days, moreover, fall just at the time that the eels are north of Scotland and would normally be turning towards Sweden.

● In the face of public and institutional protests, the mining company LKAB has withdrawn its controversial application to extract uranium from a 15-km² area around South Billigen (see *Nature*, 257, 733; 1975). It will make another application next year, after revising its plans to take more account of the need for environmental protection.

Resuscitation for advanced ground transport?

THOSE in the UK engaged on research into advanced ground transport, and in particular transport by magnetic levitation and propulsion ("maglev"), suffered a sorely-felt setback two years ago when the Conservative government decided not to continue its support for the work of Tracked Hovercraft Ltd.

If they needed a reminder of how unlucky they were by comparison with their contemporaries working years earlier on supersonic air transport—who were more fortunate in being allowed the indulgence of the "white heat of technology" era, when economic obstacles were less obvious—it came last week in a report from the Science Research Council (SRC).

The report records the reasons why a panel established by the SRC's Engineering Board rejected a request from a group of universities for support for a programme of research using the hovertrain test-track facilities at Earith, near Cambridge. The application followed a recommendation from the House of Commons Select Committee on Science and Technology that the track should be maintained as a focus for research work.

The report's main conclusion is hardly encouraging. The prospects for the adoption of maglev techniques for the "slowly evolving" urban developments, or for inter-city ground transport at speeds in excess of those of the Advanced Passenger Train (APT), are, it says, "at best highly uncertain".

One important reason for this, it adds, is "the lack of knowledge of the technology, and therefore of the likely cost and performance obtainable . . . when seen in the context of the world economic situation".

But the report's real emphasis is on the absence of an adequate body of knowledge, which was underlined by the "lack of success" of large-scale demonstrations. Moreover, "our comprehension of the theory, design and performance of the various forms of linear motor is inadequate".

The report's recommendations, however, offer the scientists more hope. The SRC, it suggests, should encourage analytical studies of linear motors and magnetic suspension systems, experimental verifications of the analyses and, significantly, assessments of the economic implications and safety requirements. And it also proposes that the panel be reconstituted to stimulate research, review the technology and examine the case for a rotation-type test facility, for which a financial commitment of £2.5 million

over five years is tentatively indicated.

Responses to the report from universities, government departments and such concerned industries as British Rail, all of whom were represented on the panel, are now awaited. Grounds for optimism must be few and far between, given the deterioration in the country's (and British Rail's) economic position since the project was dropped amid wide publicity. □

Improving outlook for tidal energy

by Allan Piper

THE slow march towards a fuller exploitation of some of Britain's more unorthodox energy resources advanced another step last week when a proposal for harnessing tidal power in the Severn Estuary received a generally favourable hearing by the House of Commons Select Committee on Science and Technology. The hearing gave leading proponents of the scheme a valuable opportunity to redress a balance that tipped against them following the recent publications of an adverse report by the Central Electricity Generating Board (CEGB), and it seems likely that the committee will have been sufficiently convinced of the project's viability to recommend that it becomes the subject of a full-scale feasibility study.

Meeting in Bristol to take evidence on the Severn Estuary scheme as part of a wider investigation into novel energy sources, the committee heard that the CEGB's main reservations about the cost of a tidal barrage were based on outdated and erroneous assumptions. The main criticism came from Dr Tom Shaw of Bristol University's Department of Civil Engineering, who challenged the estimate that the capital cost involved in the project would be twice that needed to produce an equivalent energy supply from nuclear sources using the American Light Water Reactor (LWR). He claimed that the estimate was no longer valid because recent independent estimates had put the figure at nearly double that quoted by the CEGB. The calculations have in any case been overtaken by Britain's decision not to use the LWR.

Dr Shaw, who has been involved with the project for 10 years, also contested the CEGB's suggestion that the construction of the barrage would consume more energy than would ultimately be produced. He told the committee that once the project was completed, at an estimated present day cost of around £1,500 millions, its pumped storage facility would provide for the continuous generation of elec-

tricity for little or no further capital outlay.

CEGB representatives at the hearing did not dispute Dr Shaw's claims, and the board has indicated its willingness "to cooperate in full" over the future of the scheme. Nonetheless, their apparent opposition to the project so far raises the question of whether the control of any electricity it may ultimately produce need necessarily rest with them rather than with a specially created alternative authority.

The idea of throwing a barrage across the Severn Estuary, which provides an estimated two-thirds of Britain's exploitable tidal energy, was first suggested seriously as long ago as 1924. The latest scheme involves the construction of an h-shaped barrage, the main part of which would span the Estuary from Weston-super-Mare to Cardiff. The pumped storage scheme would operate using the smaller barrier across Bridgewater Bay. Once operative it could produce about 30,000 MWh a year, which is about 10% of the present electricity demand met by the CEGB and about the same as the anticipated output from nuclear sources.

Though the figures and Dr Shaw's evidence may have gone some way towards convincing the Select Committee of the scheme's viability, several reservations remain to be dispelled before it can be given the go-ahead. Not least among these are the problems involved in the construction of the barrage, and its likely effect on the estuarine environment and local shipping movement. Last week's hearing included evidence from local civil engineering consultants, the Severn Valley Water Authority and the Port of Bristol Authority.

Though the attitudes of the SVWA and PBA are apparently not wholly favourable towards the scheme, the corresponding authorities on the Welsh side of the estuary may be less antagonistic. The artificial high tide created by the barrage, for instance, would benefit the port of Cardiff, because for the first time tankers with displacements of up to 150,000 tonnes would be able to approach to within a few hundred yards of the quayside.

Before the Select Committee closes its investigation into the project—a report is unlikely to appear much before the middle of next year—it will call the Secretary of State for Energy, Tony Benn, to give evidence. His ministry has presented him with an unfavourable report on the scheme, and the committee will be interested to learn why the project is not considered worthy of a feasibility study.

Whatever the details of the committee's eventual recommendations, supporters of the scheme should be

encouraged by the unofficial attitude of some of its members. Following last week's hearing Chairman Arthur Palmer openly admitted on British television that he was optimistic that the first part of the project could be completed and producing electricity within ten years. □

New president for Royal Society

LORD Todd has been elected President of the Royal Society for the next five years; he succeeds Sir Alan Hodgkin, president since 1970. The new president is 68, and is not entirely new to the office—his father-in-law, Sir Henry Dale, was president of the society from 1940–45.

The long list of positions he has held and honours he has received includes periods of research or lecturing at Glasgow, Frankfurt, Oxford, Edinburgh, London, Caltech, Manchester, Chicago, Sydney, MIT, and (since 1944) Cambridge. It also includes the Nobel Prize for Chemistry in 1957. From 1952–64, he was Chairman of the Advisory Council on Scientific Policy, and he was President of the International Union of Pure and Applied Chemistry from 1963 to 1965.

Sir Alan, in his retiring address, was critical of the government for its failure to appoint a chief scientific adviser to the Cabinet. "The need for central coordination", he said, "would become acute if the country were faced with a sudden crisis involving science and technology . . . the Government would have to deal with a number of awkward questions . . . which did not fit easily into the remit of one research council or ministry . . . the chief scientific adviser would know his way round Whitehall, and he would know how to get the best scientists to work, both inside and outside Whitehall." The post of Chief Scientific Adviser has been vacant since Sir Alan Cottrell returned to academic life more than a year ago. □

Reforms suggested for French Academy

from the staff of La Recherche

A report on the French *Académie des Sciences*, completed in the summer but so far not made public, has made recommendations which have already given rise to some controversy and criticism, particularly from the present academicians.

The report, from a small committee headed by P. Aigrain which was asked earlier this year by President Giscard d'Estaing to study principles and

methods of reform, consists of a series of suggestions aimed at giving the Academy a more important role and one similar to that of some other national academies.

The report says that reform of the Academy must begin with lowering the average age of members, which is currently 72. The committee studying ways and means has proposed that, on attaining the age of 70, academicians should be invited to take an "emeritus" position, and retire with a pension. The number of academicians should also be gradually increased, over a period of three years, to 175. The number of "corresponding members" would be raised to about 400, and of foreign "associate members" to about 50. This increase in the number of academicians would pose a serious problem, however, in that, if spread over only three years, it would necessitate 25 to 30 elections per year.

The Aigrain report also recommends the reorganisation of the present inadequate divisions within the Academy. It advocates the creation of two divisions, with mathematics and physical sciences grouped together, and a second group combining chemistry, the life sciences and maybe the social sciences. A fixed percentage of academicians' seats would be reserved for representatives of the applied sciences.

Other less drastic measures are also proposed: an increase in the next budget of the Academy to give it real autonomy; the election of a president every three years, with the option to renew the term of office (the current term is one year); and the creation of posts for foreign researchers invited by the Academy to spend some time in France as "Academy Professors".

Although many of the present members of the Academy think the reforms suggested are too stringent, particularly the proposals to bring in more young blood, and although they oppose the Aigrain report, the Academy's place in French society has not always remained unquestioned. Founded in 1666 on the initiative of Colbert, it played a major part in French science for more than a century. The academicians with their pensions were in some ways the first professional researchers. Instructed by the government to carry out various scientific and technical studies, they did on occasions provide a real scientific advisory service.

The Academy's influence subsequently waned, and governments since 1945 have built new mechanisms for scientific policy which have effectively removed the Academy from decision-making centres and have caused the French scientific community to question its capacity to represent them.

The Minister for Industry and Re-

search and the Secretary for State responsible for the universities must now decide on which measures to adopt. They will doubtless give in on one point at least, that the lowering of the age range could be spread over five years instead of three. However, since the Academy has elected a relatively young man, Professor P. Germain, to the post of Permanent Secretary, to replace L. de Broglie on his resignation, it appears that, even if with rather bad grace, the Academy is ready to accept a large part of the proposed reform. □

Occasionally the animal kingdom seems to decide on its own to fight back against the manifestations of modern civilisation. In northern Israel recently, Syrian woodpeckers actually destroyed kibbutz irrigation systems. Apparently, they find the sound of water rushing through plastic irrigation pipes similar to the sound made by various insects inside trees, and so peck away at the plastic as if it were wood. Because the woodpeckers did not stop after failing to discover any insects, people are wondering whether the birds have developed a taste for plastics. Although distressing for the farmers, the development has at least shown that traditional plastics may be biodegradable after all.



Stamping out pollution

Israel's environmental problems are particularly severe because of a decades-long tendency to disregard ecological considerations in an attempt to achieve rapid development at almost any cost. This now seems to be changing, if the establishment of several bodies to deal with environmental deterioration is anything to go by. A series of stamps (above) has also been issued on the subject. They deal with air pollution (a butterfly engulfed by factory fumes), water pollution (a fish swimming in waste discharged by a ship) and noise pollution (an ear, presumably human, under assault by sound waves from a jet). Tabs below the stamps show the butterfly in the company of flowers rather than fumes, the fish next to an underwater plant rather than a ship, and an ear serenaded by birds rather than jets.

from Nechemia Meyers

Peer-review under review

by Colin Norman, Washington

IN response to a mounting chorus of complaints from a few vociferous members of Congress, and background grumblings from a number of scientists, the two government agencies chiefly responsible for supporting basic scientific research in the United States have launched an important inquiry into the methods used for reviewing grant applications. The National Science Foundation (NSF) and the National Institutes of Health (NIH), which together spend \$2,500 million on research each year, have begun three separate studies of their grant-award systems. The results, which should be available next year, will probably provide at least a partial vindication of the agencies' procedures, but some fundamental principles are at stake, and some changes can be anticipated.

At the heart of the inquiry is the so-called peer-review system, which is used in some shape or form by virtually every government agency which supports academic research. Although it has never lacked critics, the system has been vigorously defended by most scientists as the fairest, and probably the best, method for judging the relative merits of competing grant proposals.

NSF handles some 21,000 grant proposals each year, nearly three quarters of which are sent out by mail to several scientists to review. A third of those are also reviewed by a panel of scientists at a meeting. Most of the rest are reviewed by a panel only, frequently with the applicant along to discuss his proposals, while a few are not peer-reviewed at all. NIH grant applications are nearly all reviewed by panels of scientists. In both cases, officials from the agencies ultimately decide which applications will be funded.

Traditional defences of the system have clearly failed to mute the criticisms. In fact, the reverse seems to have happened. Thus, NSF last month sent out 4,500 questionnaires to grant applicants and reviewers of grant proposals to gauge the feelings of the scientific community toward its grant awarding mechanisms, and it has also asked the National Academy of Sciences to undertake an independent investigation of the peer-review process. Meanwhile, NIH has established a top-level internal committee, under the chairmanship of Dr Ruth Kirschstein, director of the National Institute of General Medical Sciences, to conduct a broad evaluation of NIH's grant system.

The two agencies have been pressed

into their investigations by an extraordinary series of political attacks on basic research from congress throughout the past year. NSF was thrown on the defensive about a year ago when Senator William Proxmire, who has previously attacked the Pentagon budget, criticised a few NSF-supported research programmes which he considered to be a waste of taxpayers' money.

By publicising trivial or humorous grant titles, and their costs, Proxmire ensured wide press criticism of NSF, which in turn made people ask why Congress wasn't keeping a closer watch on NSF activities. The House of Representatives attached a provision, later dropped, to an NSF budget bill which would have given Congress a chance to vet every research project which NSF wanted to support, and to veto those which the politicians didn't like. It sent cold shivers through the scientific community.

But that was only the beginning. NSF has more recently found itself in hot water with right-wing congressmen because of its sponsorship of some controversial school science courses. Led by a conservative Republican from Arizona, John B. Conlan, the critics began by attacking the courses and railing against NSF's role in developing and promoting them. But their criticism turned into a broader attack on NSF's peer review system when Conlan was denied access to peer-review reports on one NSF course which he considered particularly offensive. He then turned his attention to the secrecy of the peer-review process which, he asserted, makes it impossible for Congress or the public to hold NSF accountable for its actions.

The matter eventually came to a head last summer, when the House Committee on Science and Technology held two weeks of hearings on NSF's peer-review process, in the course of which Conlan and others presented their thesis that peer-review, as used by NSF, provides too much power to NSF officials, stifles innovative proposals and gives rise to what Conlan described as "an incestuous buddy system". Following the hearings, Conlan and Senator Jesse Helms, a conservative Republican from North Carolina, introduced similar bills into the House and Senate calling for a radical overhaul of the system at NSF which, among other things, would ensure that verbatim copies of peer-review reports, complete with the name of the reviewer, should be made available to the applicant and to Congress.

NSF, in the meantime, has been busy preparing its defence and making some concessions. The National Science Board (NSB), NSF's governing council, has decided that, beginning in January, grant applicants should be able to

obtain verbatim copies of the reports of reviewers of their grant proposals. The NSB also decided, however, that, for the moment, the names of the reviewers should remain confidential, a policy which NSF's director, Dr H. Guyford Stever, has defended on the grounds that many scientists would be unwilling to review grant proposals if they are not guaranteed anonymity, and that open reviews would be less candid. It is partly to judge the accuracy of such assertions that NSF is soliciting the opinions of scientists concerning its review procedures.

The NSB, in conjunction with the House Science and Technology Committee, drew up the questionnaires and mailed them out to equal numbers of successful and unsuccessful grant applicants and reviewers. The results will be analysed early next year by a Congressional agency, the General Accounting Office, and they will provide the first comprehensive indication of the attitudes of scientists towards the peer review process.

In the meantime, the National Academy of Sciences' Committee on Science and Public Policy (COSPUP) has entered into a contract with NSF to conduct an independent investigation of the peer-review process. Its first report, describing the system and pinpointing its strengths and weaknesses, should be completed next summer, and a later report will deal with the manner in which the results of NSF-supported research are used.

As for NIH, it has been spared most of the recent congressional criticism of the peer-review process, but there have nevertheless been repeated grumbling about its granting mechanisms from a variety of sources. Consequently, top NIH officials decided to establish the internal review committee. Its mandate is a broad one. It has been asked to look into the philosophy behind NIH's peer-review process and to recommend necessary modifications, to examine alternatives to the system, to investigate the effects of recent legislation calling for more openness in governmental decision-making on peer-review and to look into the possibility of establishing a formal appeals process for disgruntled grant applicants.

The Committee is concerned only with the procedures used to evaluate grant proposals, not with NIH's growing commitment to contract research. It is hoping to hold three sets of public hearings in February to solicit the ideas of NIH's clients in the universities, and its final report is due by June 30 next year.

Whatever the various investigations of the peer-review process finally conclude, some matters dear to the hearts and professional well-being of the scientific community are at stake. □

news and views

Chirality and the origin of life

from Tilo L. V. Ulbricht

ONE of the unsolved problems (there are many) concerning the origin of life is the choice by living organisms of one set of optical isomers: as is well known, only L-amino acids are found in proteins, whereas laboratory syntheses from optically inactive starting materials yield 1/1 (racemic) mixtures of L- and D- isomers. One can understand how the optical purity of living organisms is maintained, once it exists, because enzymes, composed exclusively of L-amino acids, catalyse the formation of only the desired isomer. Moreover, many organisms even have a special enzyme, D-amino acid oxidase, to remove the unwanted isomer. But how did it all begin?

Could there have been some asymmetric factor in the environment which influenced the choice? It has been suggested that life began in the vicinity of optically active quartz crystals, but then the choice of L- or D- was merely a matter of chance. Another suggestion is that circularly polarised light might have been the agent. Many years ago, Kuhn showed that circularly polarised light could induce optical activity by asymmetric decomposition, and much more recently true asymmetric synthesis has been accomplished using circularly polarised light. But consideration of the interaction of the Sun's radiation with its own or with the Earth's magnetic field, and the fact of the frequent reversals of the Earth's magnetic field, indicates that there was no predominant handedness in the circular polarisation of incident sunlight over any significant time spans.

A further possibility is an asymmetry in the environment from natural radioactivity. There must have been quite a few scientists who were struck by the analogy between the asymmetry at the level of elementary particles (left-handed electrons and right-handed positrons) and asymmetry at the molecular level, when the discovery was announced in January 1957 that parity was not conserved in β decay. But the existence of the analogy does not mean that any causal relationship exists. Vester and I examined this possibility, suggested some mechanisms and early in 1957 carried out

experiments to try and induce optical activity in chemical systems under the influence of polarised (left-handed) β rays. (For a review of the earlier literature, see Ulbricht, *Origins of Life*, 6, 303; 1975.) One of the mechanisms proposed was the following: longitudinally polarised β rays $\rightarrow$ circularly polarised bremsstrahlung $\rightarrow$ optically active molecules.

As had been predicted by Lee and Yang, the ultraviolet radiation produced when β rays slow down in matter (bremsstrahlung) is circularly polarised if the β rays are polarised. Thus, at least in principle, a route from polarised electrons to optical activity exists. The early experiments, however, were negative or inconclusive, although in retrospect one result was interesting. This was an experiment in which D, L-alanine was mixed with Ag powder and irradiated in a neutron pile, conditions in which the radioactive isotopes ^{108}Ag and ^{110}Ag are formed. Ninety-six per cent of the alanine was destroyed, and the soluble residue had a very small rotation which was not considered significant.

Because of these inconclusive results and the, as it then seemed, rather far-fetched nature of the hypothesis, there was little interest in the idea for some years. Two quite independent factors stimulated new activity. The first was the realisation by physicists that parity violation occurred not only in weak interactions like β decay (which have little to do with chemistry) but that there is also a small parity-violating component in all electromagnetic interactions—which are, of course, at the very basis of chemistry. This was strengthened by the discovery of neutral currents, suggesting that weak and electromagnetic interactions may have a common foundation. One consequence of this is the realisation that optical isomers do not have exactly equal energy contents. Some experiments designed to amplify this small difference have yielded positive results (see Ulbricht, *op. cit.*). The second factor was a paper by Garay (*Nature*, 219, 338; 1968) reporting that solutions of D- and L-tyrosine decompose at different rates in the presence of β rays from ^{90}Sr -Y. This was the first

evidence that polarised electrons could distinguish between L- and D-isomers.

Vester and I had thought it unlikely that a direct interaction between high-energy electrons and molecules could produce optical activity, and similarly, Ulrich and Walker (this issue of *Nature*, page 418) argue that stereospecific discrimination is unlikely in high energy processes. They think, therefore, that if asymmetry from β rays is communicated, it must be by way of the secondary electrons created during their energy degradation. Accordingly, they have searched for evidence of optical activity in solvated electrons, supposing that an electron confined to a helical solvent may show a different probability of reaction with D and L isomers. No evidence of such a difference was found, or of optical activity of solvated electrons in an optically active solvent. These negative results would seem to rule out certain mechanisms involving secondary electrons, though it should be noted that differences in rate constants of less than 10% could not have been detected.

However, several recent investigations have produced positive results. Kovacs and Garay (*Nature*, 254, 538; 1975) did a large series of crystallisations of D,L-sodium ammonium tartrate in the presence and absence of ^{32}P -phosphate, measuring the optical activity of the crystals. Whereas a virtually symmetrical distribution of optical activity about zero was obtained in the control series, in the presence of ^{32}P the distribution was markedly asymmetric, the L-salt crystallising preferentially.

In view of the doubts about some of the previous experiments in this field, the results of Bonner *et al.* (page 419, this issue) will attract particular attention. Using a special apparatus which could produce either left-handed ("natural") or right-handed ("unnatural") electrons of 10–23% polarisation, solid D,L-leucine was irradiated to 50–75% decomposition. Determination of the enantiomeric composition of the undecomposed leucine residue showed that the left-handed electrons (those having the same anti-parallel spin as natural β rays) brought about

the degradation of D-leucine more than of L-leucine; and what is most striking, right-handed electrons decomposed L-leucine more than the D-isomer. Thus, the "natural" electrons produced an asymmetric degradation favouring the "natural" amino acid, and *vice versa*.

From the results reported in the last few months it seems reasonable to conclude that β rays can indeed induce optical activity. What remains totally obscure is the mechanism; for example, in the experiments by Bonner *et al.*, was the effect a direct one of the polarised electrons themselves, or was it due to bremsstrahlung? □

Transposable resistance genes

from J. R. Saunders

It is increasingly evident that certain bacterial resistance genes are capable of migration between DNA molecules. This ability makes it easier to understand the rapid evolution of antibiotic-resistance (R) plasmids possessing varied combinations of linked resistance genes and may indicate a more general evolutionary process in bacteria and other organisms.

The curious ability of a gene specifying ampicillin-resistance to be translocated to different replicons in bacteria has been known for some time (see, for example, Anderson, *CIBA Symposium on Bacterial Episomes and Plasmids*, 102, 1969; Richmond and Sykes, *Genet. Res.*, **20**, 231; 1972). Hedges and Jacob (*Molec. gen. Genet.*, **132**, 31; 1974) demonstrated that the ampicillin-resistance gene present on the plasmid RP4 can be acquired by a variety of other plasmids co-existing in the same strain of *Escherichia coli*. Plasmids acquiring this gene showed concomitant increases in molecular weight, and were in turn able to translocate the gene, which they termed Transposon A (TnA), to other plasmids. Recently a number of other resistance genes capable of translocation between chromosomal, phage and plasmid DNA have been identified. These include, for example, kanamycin-resistance (Berg *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3628; 1975), tetracycline-resistance (Kleckner *et al.*, *J. molec. Biol.*, **97**, 561; 1975) and linked resistance to streptomycin and trimethoprim (Hedges *et al.*, *Mol. gen. Genet.*, **140**, 289; 1975). This suggests that discrete translocatable elements (transposons) may constitute relatively common genetic units in bacteria.

Transposons are apparently able to

insert into and excise from DNA molecules in the absence of a functional bacterial recombination (*rec A*) system. This suggests that such elements use alternative, possibly self-specified enzymes for recombination. Despite this partial independence, transposons have not been shown to exist autonomously and need to reside in a functional replicon.

A clue to the mechanism of insertion and excision comes from electron microscope studies of DNA heteroduplexes formed between phage or plasmid DNA with and without inserted transposons. These indicate that most transposons are composed of a resistance gene (or genes) flanked on either side by inverted complementary DNA sequences (inverted repetitions) of between 100 and 1,500 nucleotide pairs. Some, but not all such repetitions are homologous with known insertion sequences (IS). For example, the inverted repetitious sequences bounding the transposable tetracycline-resistance gene are homologous with IS3. (Ptashne and Cohen, *J. Bact.*, **122**, 776; 1975). Insertion sequences are present at various sites in both plasmids and the chromosome of *E. coli* (Hu *et al.*, *J. Bact.*, **122**, 764; 1975) and have been implicated in a number of *rec A*-independent and illegitimate recombination events.

Insertions of transposons are mutagenic when occurring in structural genes and polar when occurring within operons. This is also a general property of insertion sequences (Starlinger and Saedler, *Biochimie*, **54**, 177; 1972). As shown by Klechner *et al.* insertion itself is a relatively precise process which does not damage the informational capacity of host DNA. On the other hand, excision often results in permanent loss of host genetic information. Heffron and colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 3623; 1975) show that TnA is able to insert at a minimum of twelve distinct sites on the genome of the plasmid RSF1010, suggesting recognition by the transposon of a specific but fairly common nucleotide sequence at or near to the point of integration. Insertion/excision must be by a mechanism which conserves the inverted repetitions since transposons can be repeatedly translocated whilst retaining such sequences. This could occur if excision involves recombination between the two flanking inverted repetitions such that one of the 'arms' of the repeat is retained by the genome and the other by the looped-out resistance gene. The latter could then circularise and migrate either to another DNA molecule or to a different site on the same molecule. Provided that a homologous repeat sequence is present on the recipient

molecule insertion is then possible by recombination between the two repeats. It should be pointed out however, that circularised transposons have not yet been detected in the process of migration, which suggests that such a stage, if present, is short-lived.

The significance of transposition is that it allows rearrangement and recombination of certain genes from heterologous genetic backgrounds. The phenomenon also provides a neat mechanism for the accretion of resistance genes by sex factors to form infectious multiple resistance plasmids. It is known that relatively few types of resistance gene are common to a wide variety of R plasmids, found in different bacterial species, and believed on both genetic and physical grounds to be of disparate evolutionary origin. For example, the β -lactamase (penicillinase) gene of TnA, and base sequences homologous with it, are widely distributed on an assortment of plasmids (Heffron *et al.*, *J. Bact.*, **122**, 250; 1975). This suggests that all have received the resistance gene from a common source. Transposons presumably evolved initially in their ancestral host by association of resistance genes with inverted repetitious sequences. Such elements could then be attached to a variety of replicons, including genetic vectors and hence find their way to other bacterial strains where they could be retransposed to resident DNA molecules. This process is of enormous evolutionary advantage because it obviates the need for the gross genetic homology normally required for recombination and allows reassortment of genes on different replicons. Therefore tandem insertion of numbers of resistance genes into a plasmid of unrelated nucleotide sequence would be possible, provided that the relatively short relevant inverted repetitions were present.

Transposons may prove powerful tools for genetic research since attachment of a resistance gene to a DNA molecule provides it with a readily discernible genetic and physical tag. It will be of great interest to discover the precise mechanism of their insertion and excision. Ways of doing this would include sequencing of the inverted repeats, particularly at their functions with non-repetitious DNA, and searching for a specific enzymatic machinery for mediating insertion and excision. It seems likely that the latter will involve enzymes with the specificity of restriction nucleases for recognising insertion sites. In view of the frequency of inverted repetitious DNA in both prokaryotes and eukaryotes, transposons may represent a more general class of genetic elements contributing greatly to evolutionary flexibility. □

Classical interferometry and quantum statistics

from J. Byrne

IN the half-century since the advent of de Broglie's theory of matter waves many striking and beautiful demonstrations have witnessed to the truth of this hypothesis. Under various shapes and guises the classic experiments of Young and Fresnel have been reproduced with particle beams, the motivation, at least in part, being the desire to determine the limits within which these observations may be encompassed by the framework of a classical wave field. Experiments which differentiate between classical and quantum wave phenomena are rare exceptions, and the interest they excite is correspondingly greater. A recent variant (Werner *et al.*, *Phys. Rev. Lett.*, **35**, 1053; 1975) of Young's double slit experiment, which establishes in a direct and unambiguous way the fermion character of neutrons, falls squarely in the rare category—a new departure in a field with a long and distinguished history of innovation.

Angular momentum algebra, the language of rotations, is not a topic which normally rates a mention in treatises on interferometry, but in the present context it provides a useful starting point. The notion of a vector is familiar in classical physics as an entity, which, having magnitude and direction, is characterised by three real numbers. In quantum systems of spin S the number of components is $2S + 1$ and by this criterion vector fields have unit spin. However S is also permitted to take half-integral values; this brings in spinor fields of which two component systems are the most prominent. The spin-statistics relation, which associates integral spin with bosons and half-integral spin with fermions, rests on the firmest of theoretical and experimental grounds.

For the operation of rotation to have a meaning for spinors the components are necessarily complex; the additional degree of freedom implies that spinors may be assigned a phase in addition to their magnitude and direction. An unavoidable consequence is that spinors undergo a sign change under rotation through an angle of 2π about any axis, the property which is of current experimental interest.

Uncharged and having a small magnetic moment, the neutron represents an ideal fermion system in which to detect the phase difference between coherently superposed spinor fields which have been subjected to a relative rotation. (Bernstein, *Phys. Rev. Lett.*, **18**, 1102; 1967; Aharonov and Susskind, *Phys. Rev.*, **158**, 1237; 1967). In a magnetic field the two

spin states have energies $\pm \frac{1}{2}\hbar\omega_L$, where ω_L is the angular frequency of Larmor precession. Because the force field is conservative the magnetic energy is compensated by a change in kinetic energy and the free spinor

$$U_0 = \begin{pmatrix} a_+ \\ a_- \end{pmatrix} \exp(ip_0 x/\hbar)$$

is transformed in a magnetic field to

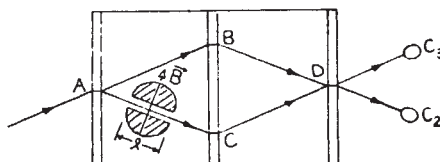
$$U_H = \begin{pmatrix} a_+ \exp(i\sqrt{(p_0^2 - m\hbar\omega_L) \cdot x}) \\ a_- \exp(i\sqrt{(p_0^2 + m\hbar\omega_L) \cdot x}) \end{pmatrix} \\ \approx \begin{pmatrix} \exp(-i\omega_L x/2p_0) & 0 \\ 0 & \exp(-i\omega_L x/2p_0) \end{pmatrix} U_0$$

to first order in $\hbar\omega_L/(p_0^2/2m)$. The transformation matrix represents a rotation of the spinor through an angle $\phi = m\omega_L x/p_0$.

The coherent superposition of U_H and U_0 results in the spinor $U_C = U_H + U_0$ for which the intensity is

$$I = |U_C|^2 = |U_0|^2 = 4(1 + \cos^2(\phi/2))$$

The minimum at $\phi = 2\pi$, which corresponds to a shift of half a fringe in the interference pattern as U_H rotates through an angle of 2π , is a direct consequence of the two-valued nature of spinors. Such an effect would not be observed for vector particles such as the metastable ortho-helium atom.



A schematic diagram of the neutron interferometer. On the path AC the neutrons are in a magnetic field B (0 to 500 G) for a distance l (2 cm).

The very simplicity of these ideas tends to mask the formidable technical problem presented by a laboratory investigation in which the combination of spatial coherence in the two beams with total spatial separation is of the essence. The system adopted by the experimenters, and illustrated in the figure, bears some resemblance to an optical interferometer of Mach-Zehnder type. A symmetrical Laue reflection at B gives two coherent beams with an angular separation of twice the Bragg angle; following further reflections in the mirror BC the beams are recombined in the analyser AD . All elements of the interferometer are formed by cutting grooves in a single dislocation-free crystal of silicon. One of the component beams propagates normal to a magnetic field and the resultant interference pattern is observed by recording the counting rates at C_2 and C_3 . The crucial advantage which the interferometer enjoys over its optical counterpart stems from the use of diffraction for

purposes of amplitude division which, for small Bragg angles, results in an achromatic system of fringes (Bonse and Hart, *Appl. Phys. Lett.*, **6**, 155; 1965). In these circumstances the coherence length is effectively infinite and the Laue technique may be employed in a broad band of neutron energies.

To conclude, two brief observations may be made. First, the rotation of the spinor U_H may be established experimentally by measuring the polarisation of those neutrons which traverse the magnetic field. On the other hand the interference effect is observed with the spinor U_C and for these neutrons the polarisation is turned through an angle of π not of 2π . There is therefore no question of making simultaneous observations of the spin rotation and of the phase difference. The second comment refers to the unitary property of the rotation matrix for spin $\frac{1}{2}$ fermions which implies that an equivalent interference effect should be observable for any two-state system, fermion or boson, which is taken through a full cycle of unitary transformations. It must be emphasised however that only in the case of fermions can such a cycle of change be understood as a spatial rotation. $\square$

Canary mantle plume unlikely

from Peter J. Smith

SO much attention has been given to the Hawaiian-Emperor island chain in connection with the mantle plume-hot spot hypothesis that there is a tendency to forget the other areas in which similar ideas may be put to the test. One example is the group of seven Canary Islands. Not that the Canaries have been overlooked entirely. Morgan (*Nature*, **230**, 42; 1971), for example, located a mantle plume beneath the islands; Burke and Wilson (*Nature*, **239**, 387; 1972) suggested that the islands were formed over a plume active for the past 22 million years; Wilson (*Tectonophysics*, **19**, 149; 1973) classified the Canaries among "young hot spots on ocean floors that may be stationary"; and Schmincke (*Geol. Soc. Amer. Bull.*, **84**, 634; 1972) concluded that although there were insufficient data to confirm a hot spot origin, there was nevertheless some support for it.

But in spite of this interest, Anguita and Hernan (*Earth planet. Sci. Lett.*, **27**, 11; 1975) claim that, with the possible exception of Schmincke, no one has really examined the nature of the Canary island chain in any great detail. Certainly it has not been subjected to anything like as much study as the Hawaiian islands. The unfortunate result is that the Canary group "has been incorporated to the geo-

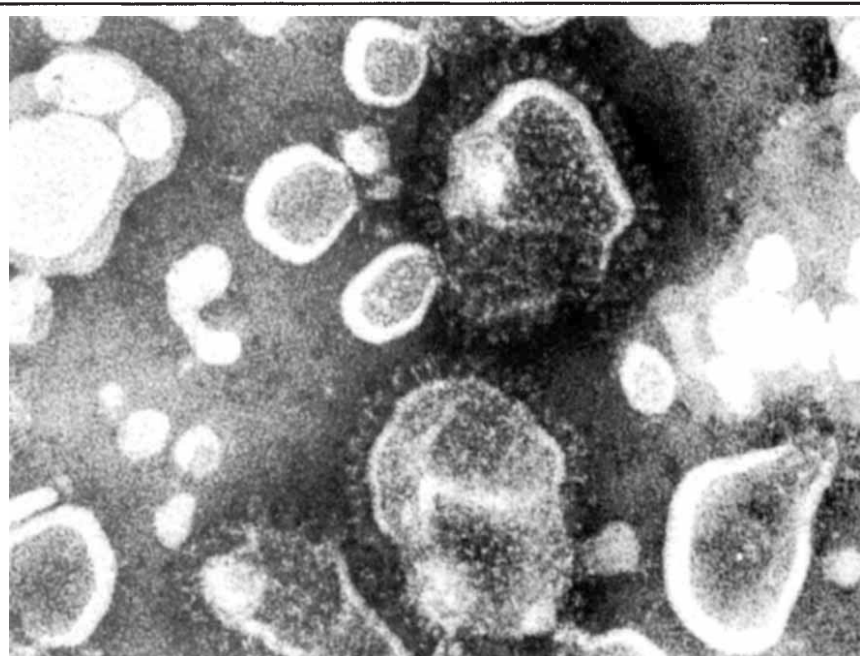
logical literature [as an example of plume activity] after little or no discussion of the chronological, bathymetric, petrological and tectonic data". Anguita and Hernan have therefore examined such information, where it exists—and conclude that the Canaries are probably not the result of plume activity at all.

Superficially, the Canary Islands qualify as a potential plume effect. The trend of the seven islands is in the direction of the local seafloor spreading and the radiometric ages of the oldest measured subaerial volcanics on each island decrease generally from one end of the chain to the other. But under closer examination the oldest-age data tell a less convincing story. First, they are not necessarily complete. The deepest levels of the exposed outcrops may not represent the earliest subaerial activity; and in one island (La Palma) the oldest dated samples do not even come from the deepest subaerial section. Second, although seafloor spreading rates quoted for the eastern Atlantic generally lie between 1 and 2 cm yr⁻¹, the apparent plate velocities calculated from the different pairs of islands in the Canaries (assuming any implied plume to be stationary) range from 0.6 to 26.7 cm yr⁻¹.

Another difficulty is that the full range of available age data (that is, not just those from the oldest subaerial flows) indicate a hiatus of 3–8 Myr in the subaerial volcanic activity on each island. In short, the subaerial volcanism is split into two distinct episodes: the first, which follows the submarine phase, and the second, which follows the hiatus.

An important feature of the three phases (one submarine, two subaerial), however, is that a trend towards greater alkalinity with time is observed within each. In the case of Hawaii, a similar (single) trend has been interpreted in terms of progressive movement away from a melting zone and thus in terms of a mantle plume beneath a moving plate. But the existence of three separate geochemical cycles appears to make nonsense of such a concept as applied to the Canary volcanics, except in the highly unlikely event of oscillating plate motion.

So if the Canaries are not derived from a plume, what is their origin? Anguita and Hernan clearly favour a propagating fracture of the sort proposed by McDougall (*Nature*, **231**, 141; 1971) and caused by the tensions implicit in the "membrane tectonics" of Turcotte and Oxburgh (*Nature*, **244**, 337; 1973). The basic idea is that a developing lithospheric fracture, tapping the asthenosphere, will allow volcanism to spread along it until the existing stresses are released. In the



VIROSOMES—Surface subunits of the influenza virus attached to unilamellar liposomes (Almeida *et al.*, *Lancet*, **ii**, 899; 1975). Anti-influenza serum aggregates the virosomes, indicating that most of the original binding sites are still available. The authors point out that virosomes may be of use in solving some of the problems asso-

ciated with vaccination against influenza. The whole inactivated influenza virus, although a good immunogen, is pyrogenic, but isolated subunits, although non-pyrogenic, are not good immunogens. Preliminary experiments indicate that the liposomes may provide the required adjuvant without pyrogenicity ($\times 275,000$).

case of the Canary Islands, however, the fracturing process would have to be repeated several times and fracturing would propagate with a non-uniform velocity.

Such a model seems to be consistent with tectonic trends (in both space and time) in West Africa. The directional trend of the western and central Canary Islands aligns more or less with the continent South Atlas Fault, a circumstance which leads Anguita and Hernan to suggest that the young oceanic fracture reflects the influence of the older system. Moreover, the western High Atlas structures of Africa were generated largely in three important orogenic pulses (in the Upper Eocene, the Upper Oligocene and the Miocene, respectively) which correlate well in time with the three distinct phases of activity in the Canaries (with each compressive pulse preceding a magmatic phase). Anguita and Hernan thus propose that it was the tensional period between each compressive pulse which allowed the build-up of stress and hence the failure of the lithosphere and the consequent upwelling of magma. Similar arguments may be adduced for the western Canary Islands, except that the directional trend involved here is slightly different.

Ultimately, however, the most in-

teresting, if not crucial, evidence against the plume hypothesis may prove to be the least direct. There has recently been much talk about possible stationary states of Africa. A proven stationary Africa and a simultaneously moving volcanic centre in the Canaries would presumably rule out mantle plumes, or at least fixed plumes, for good.

Protein structures without heavy atoms

from David Blow

UNTIL recently all detailed knowledge of protein structure came from crystallographic studies using the isomorphous replacement method. This requires a laborious screening of crystals soaked in a variety of heavy metal solutions, to see whether any provides a suitable derivative. Techniques for direct structure determination by analogy with another protein of known structure were worked out in the sixties, but had not been used successfully, and their usefulness was doubted. These doubts are finally destroyed by the re-determination of the structure of trypsin by Bode, Fehlhämmer and Schwager (*J. molec. Biol.*, **98**, 683, 693; 1975).

Previous attempts along these lines had produced little new information because of the limited resolution. Seal myoglobin and lamprey haemoglobin were shown to be close structural relatives of sperm whale myoglobin. Slightly more information was gained about carboxypeptidase B, which was shown in a study at 5.5 Å resolution to lack electron density at the N-terminus by comparison with carboxypeptidase A, which has four extra N-terminal amino acids (Schmid *et al.*, *J. molec. Biol.*, **84**, 97; 1974).

Although these methods served to confirm that one structure was like another, they were unable to provide detailed information about the new structure. Bode *et al.* show such information can be gained if one works at sufficiently high resolution.

The Munich group had already worked out one structure involving the trypsin molecule. This was the complex of pancreatic trypsin inhibitor with trypsin (Huber *et al.*, *J. molec. Biol.*, **89**, 73; 1974). They used isomorphous replacement to give an electron density map, and then used a computer method to orient the inhibitor structure, which they had worked out separately, to give the best fit to the map. For the trypsin part of the complex, a previously determined trypsin structure did not fit well, but the coordinates for chymotrypsin, edited to account for known chemical differences, could be rotated to a correct orientation and refined. During refinement, the phase angles obtained

Brightly shines the X-ray quasar

from John Gribbin

A NEW candidate for the title "intrinsically the strongest X-ray source known" has been put forward by Schreier *et al.* on the basis of observations made by the Astronomical Netherlands Satellite (ANS) and a timely revision of an old Uhuru error box (*Astrophys. J. Lett.*, **201**, L137; 1975). If the identification is correct, and if the QSO NAB0137-01 which is the subject of the identification is at the cosmological distance implied by its redshift of 0.33, then the source radiates about 2×10^{46} erg s⁻¹ at X-ray frequencies, four times as much as 3C273 and 50 times as much as its own optical luminosity.

But there must remain some doubt about the identification of the Uhuru source 3U0138-01 with this QSO, even though the evidence is suggestive. A tentative identification had already been made on the basis of the original Uhuru data, and this now looks better since the Uhuru error box first published turns out to have been too short in one direction; the stretch in the box implied by subsequent revisions brings one edge almost out to include the QSO. The strip of sky scanned by ANS definitely includes the QSO, but the triangular error box resulting from a combination of Uhuru and ANS observations still does not quite include this object at the 90% level.

The QSO was originally found by Bahcall *et al.* (*Astrophys. J. Lett.*, **199**,

L9; 1975) during an optical search of the sky near 10 Uhuru high latitude sources. They estimate that 0.7 QSOs would be found among the 10 boxes by chance, and the location of NAB0137-01 just outside the 90% error box for 3U0138-01 seems to fit this estimate rather well. Schreier *et al.*, however, point out that with the ANS data this particular error box is now much smaller, and say that the statistical chance of finding Bahcall *et al.*'s 0.7 of a QSO in this particular box out of the 10 is much smaller. The combined ANS/Uhuru box covers 0.02 square degrees, while the other nine Uhuru boxes of this search range from 0.14 to 0.52 square degrees in size. The probability that a QSO would be found by chance in a box of 0.02 square degrees is only 0.005; but the statistical argument remains unsatisfactory, as is usually the case for samples of one, and further identifications of high latitude X-ray sources with QSOs are needed before this can be taken at face value.

Even so, the possibility that NAB0137-01 has such a large X-ray to optical luminosity ratio is interesting in view of the lack of other identifications. It suggests that the optical objects associated with such X-ray sources may be very faint, and highlights the need for searches down to limiting magnitudes of at least 18—as well, of course, as emphasising the need for better X-ray positions.



THERE has just been placed in Westminster Abbey a marble scroll bearing an appropriate inscription to the memory of Jeremiah Horrocks. The movement for such a memorial was commenced some time ago, and is referred to in *NATURE*, vol. x. p. 190, and xi. p. 31. The inscription is as follows:—

In Memory of
JEREMIAH HORROCKS,
Curate of Hoole, in Lancashire,
Who died on the 3d of January, 1641,
in or near his 22d year;
Having in so short a life
Detected the long inequality in the mean
motion of Jupiter and Saturn;
Discovered the orbit of the Moon
to be an ellipse;
Determined the motion of the lunar apse;
Suggested the physical cause of its revolution;
And predicted from his own observations
the Transit of Venus,
Which was seen by himself and his friend
William Crabtree
On Sunday the 24th of November (O.S.) 1639:
This Tablet, facing the Monument of Newton,
Was raised after the lapse of more than
two centuries. December 9, 1874.
from *Nature*, 13, 112, Dec. 9, 1875.

from isomorphous replacement were abandoned in favour of phase angles calculated from the partly refined structure.

This is the key point of the whole operation. At lower resolution, the insertion of calculated phase angles would firmly fix the structure at that assumed in the phase calculation. This work was done at 2.3 Å resolution, where there is sufficient detail in the electron density map to require every atom to be in the right place.

In the latest work, the trypsin structure obtained from the complex was used as a starting point. Instead of isomorphous replacement, a search was made for the best orientation of the trypsin molecule to fit the diffraction data. This orientation was used to provide calculated phases, and the procedure continues as before, this time at 1.8 Å resolution.

The results show a remarkable consistency. The two trypsin structures have a mean difference of 0.25 Å (though due to error in the reorientation of the trypsin molecule, the start-

ing orientation averaged 0.44 Å away). Main chain atoms common to chymotrypsin and trypsin differ on average by 0.75 Å, and most side chains and internally bound water molecules are in similar conformations. Considering the different conditions, and the fact that these comparisons must include experimental error, they indicate that the polypeptide chain of these enzymes has evolved considerable rigidity.

Working at a lower resolution of 3.5 Å, Ward *et al.* (*J. molec. Biol.*, **98**, 161; 1975) have determined the structure of new crystal forms of human deoxyhaemoglobin A and S, using similar techniques. At this resolution no refinement was possible, and the authors are rightly cautious in interpreting apparent differences between their structures (crystallised in polyethylene glycol solutions) and the normal form. The particular interest of these structures is that they may provide a clue to the mechanism by which sickle cell haemoglobin aggregates into fibres.

These techniques mean that a new

avalanche of protein crystal structures is on its way, hastened by the automatic techniques now available for data collection. Travellers to the East Coast Protein Crystallography meeting in November returned dazed by the overwhelming tide of information, including one structure solved at 3.5 Å resolution, start to finish, since April. But there are many fewer structures which have been determined accurately and pondered deeply. It looks as if these direct methods are only going to give new structural information if they are taken to resolutions beyond 2.5 Å. Even with automated equipment, that comes fairly slowly, and carries with it a hefty computing bill.

Health physics instruments, their design and calibration

from R. W. Clarke

A meeting, sponsored by the British Radiological Protection Association and presented by the Society for Radiological Protection was held on October 22 and 23 at the Berkeley Nuclear Laboratories, CEBG.

THERE is seldom a meeting involving any aspect of radiological protection where the basic units of dose measurement are not once again debated, with new systems proposed and old ones denigrated. It is not surprising therefore that this meeting opened with a valiant attempt by H. J. Dunster (National Radiological Protection Board, Harwell) to rationalise current practice by the examination of the objectives of such measurements. He declared that dose equivalent index (defined as the maximum radiation dose, corrected for quality and other factors, within a 30 cm diameter sphere consisting of material equivalent to soft tissue with a density of 1 g cm^{-3}) was the only environmental quantity for general application. It should be measured at depths in this sphere such that the superficial tissue

was 7 mg cm^{-2} and 1 g cm^{-2} (that is, 0.007 cm and 1 cm). At such depths, corresponding to the doses to skin and internal organs respectively, the result would include the dose to the eyes at 300 mg cm^{-2} . In uni-directional fields, instruments could be made to measure this index accurately or, more importantly for regulatory purposes, reproducibly. It is clear that in multi-directional fields the answers could be commonly a factor of two too high or more but this should be acceptable. More precise measurement of the radiation requires a detailed research examination of fluence and energy quality only justified for post-accident enquiries and similar cases.

Despite the development of new definitions of radiation dose, manufacturers seem to persist obstinately in producing instruments that read in the old exposure units (e.s.u. generated in air) measured in röntgens even though protection regulations are couched in terms of absorbed dose (erg per g of air) measured in rads or rems. D. White (NRPB) while appealing for the cessation of exposure meters, recommended the measurement of absorbed dose in air beneath layers of 300 mg cm^{-2} and 700 or 800 mg cm^{-2} . To those who search for ever more constant energy response in radiation detectors, he demonstrated that if the errors of individual variables, such as angular response, linearity and scales are compounded then a factor of two is arrived at for the accuracy to 95% confidence limits. There seemed little point in concentrating on the improvement of energy response which is only one of many contributing sources of error.

To the simple participant at this meeting, it seems we find ourselves still debating new units and methods of measurement while the actual users and even the national standards remain addicted to the exposure units originally defined half-a-century ago although there is common acceptance that absorbed dose in air will eventually be registered on every meter. These uncertainties are not shared by the instrument designers. While others argue dose definitions, the electronics engineer is eagerly exploiting the contemporary revolution in integrated circuits. Amplifiers, power units, discriminators and digitisers are all less than match-box size. Complete dose-meters can be less than pocket-book size. If only the radiation detectors could similarly shrink.

F. H. Wells (UK Atomic Energy Authority, Harwell) was enthusiastic on the subject of the new generation of digitised instruments where $1.5 \mu\text{rad}$ response is converted to a pulse that can be transmitted over long cables with small power requirements. Port-

able instruments with digital displays are now available for those health physicists modern enough to abandon meters and in the near future dose-meters with micro-processor devices could do all the calculations one might desire. In theory at least, the electronics can produce readings from a dosimeter in any form or units required but a salutary reminder of production costs and quantities to cover development costs was delivered by a manufacturer (S. Donaldson, Eberline).

An example of the high costs of meeting the requirements of the protection regulations for instrument calibration was provided by the electricity generating industry (I. M. G. Thompson, CEBG) which has funded a neutron-producing accelerator, X-ray machines and a multitude of standard isotope sources purely for this purpose. Yet despite this expenditure, there is at present only a tenuous path connecting health physics dosimetry to the national standards of exposure or dose. Reductions of several orders of magnitude are involved in the most common gamma dose measurements. Strenuous efforts are being made by the custodians of the national standards to produce the balloon ion chamber which has been designed to cover the health physics range of measurements (B. Owen, National Physical Laboratory, Teddington). The British Calibration Service is being used to organise and approve a chain of secondary calibration laboratories to transfer the national dose standards to the instrument user. Criteria are soon to be published for the management, equipment and methods for calibration laboratories but quotes from these criteria presented a daunting glimpse of qualified personnel and expensive equipment which few industries could support. It is clear that there will be only a few secondary laboratories.

J. H. Griffiths (UKAEA), speaking for the user, injected a shock of realism by a simple demand for reliability. There was not an anxiety over dose units nor even accuracy but a concern over the prevalence of failures and false alarms. More than one or two false alarms each year can lead people to ignore installed instruments altogether.

Thus the conflict in health physics dosimetry was complete with the purists defining yet new concepts for the measured units while the practical men were still trying to establish such requirements as calibration standards and reliability based on units of the last decade. Even this well-attended meeting could not resolve these problems but most people could at least see the confusion more clearly. □

Erratum

In the article "Histocompatibility antigens and immunoglobulins?" (*News and Views*, 258, 193; 1975), a printing error in the penultimate paragraph resulted in an omission. The first sentence of this paragraph should read "If the antigen-detergent complex does not contain a four-chain structure then there is the difficulty of explaining the reason for the appearance of dimers of the 45,000 chain in SDS polyacrylamide gel electrophoresis."

review article

The place of the australopithecines in human evolution: grounds for doubt?

C. E. Oxnard*

Although most studies emphasise the similarity of the australopithecines to modern man, and suggest, therefore, that these creatures were bipedal tool-makers at least one form of which (Australopithecus africanus—"Homo habilis", "Homo africanus") was almost directly ancestral to man, a series of multivariate statistical studies of various postcranial fragments suggests other conclusions. Their locomotion may not have been like that of modern man, and may, though including a form or forms of bipedality, have been different enough to allow marked abilities for climbing. Bipedality may have arisen more than once, the Australopithecinae displaying one or more experiments in bipedality that failed. The genus Homo may, in fact, be so ancient as to parallel entirely the genus Australopithecus thus denying the latter a direct place in the human lineage.

AN important part of today's conventional wisdom about human evolution is based on studies of teeth, jaws and skull fragments of australopithecine fossils. That these fossils are undoubtedly closely related to the human lineage is now engrained into textbooks¹⁻³. So close is this relationship judged to be^{4,5} that some of the fragments have been placed in the genus *Homo* itself (for example, species "*habilis*", "*africanus*") while others are accepted as parallel but nevertheless very close evolutionary relatives. Some investigators⁶ have long disagreed with these viewpoints on the basis of biometric studies comparing living forms and fossil fragments; more recently other workers⁷ have also begun to question accepted views because of new finds in Africa.

As postcranial bones referable to australopithecines are described it is perhaps inevitable that, believing relationships to man to be rather close, those particular features in which the postcranial bones resemble man are most emphasised. Thus those parts of the Sterkfontein pelvis which are similar to those of man (the shape of the iliac blade for instance) are a major factor in the claim that these creatures have an essentially human pelvis with a totally⁸ (or somewhat less than totally, depending on the worker⁹) human pattern of bipedal walking. Individual ankle bones (the talus) from Olduvai and Kromdraai have been assessed as indicative of established bipedal walking¹⁰ even though they differ from man more than the African apes do¹¹. A series of associated foot bones from Olduvai has been reconstructed into a form closely resembling the human foot today¹² although a similarly incomplete foot of a chimpanzee may also be reconstructed in such a manner. Hand bones, also from Olduvai, possess a total of seven features in which they resemble various apes, compared with only three in which they resemble man, but have nonetheless been evaluated as capable of tool-making in a human fashion¹³; this last evidence is now even weaker because of the relatively newly discovered

abilities of various apes for using and making tools¹⁴. A fragment of scapula described many years ago as being rather more like that of the orang-utan than anything else¹⁵ (and this has been confirmed by a recent study¹⁶) is nevertheless generally treated in discussion as if it were essentially human.

Questions therefore arise. Do the various postcranial fragments of different australopithecines provide evidence of similarity to man? To what extent, whatever the similarity, are they telling us about biomechanical efficiency for their various biological functions rather than about genetic relationships? Through the initial stimulus and investigations of Professor Lord Zuckerman, through collaboration with him and with Professor E. H. Ashton, Mr T. F. Spence and other colleagues in the Department of Anatomy, University of Birmingham (of which Lord Zuckerman was originally the Head), through collaboration with Professor F. P. Lisowski of the University of Hong Kong, and from studies in my own laboratory in the Department of Anatomy, the University of Chicago, we have used the multivariate morphometric approach to make a series of comparisons between a small number of individual fossil fragments and the equivalent bones both of living men and of a broad range of extant non-human primates^{17,18}.

The core of the multivariate approach is the following: if we suppose that a single object can be defined by three variables then that object can be represented as a point within a three-dimensional coordinate system. A group of similar objects will then appear as a tightly knit cloud or swarm of points within that three-dimensional space. Other different groups of objects will appear as other clouds or swarms which may or may not overlap with the first. If the original variables defining the objects are not correlated with one another then the original coordinate system will best describe the arrangement of the swarms. If, however, the original variables describing the objects are correlated with one another to some or other degree (the usual situation) then the true relationships among the clouds will best be seen from the vantage point of some other set of co-ordinate axes. Geometrically, this is the equivalent of

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constructing from one viewpoint a model of the clouds and then rotating the model and scanning it from a new position that gives a view best separating the clouds. If we extrapolate such a three-dimensional geometrical description to an example in which we have taken many variables on many groups then the problem is multi-dimensional and can only be solved algebraically. An equivalent to building the model and rotating it can be carried out through the manipulations of matrix algebra and using a computer¹⁹.

Although multivariate statistical approaches have been used by many workers in different anatomical regions and in different groups of animals and plants for characterising and comparing complex shapes, in our laboratory they are used in a rather special manner for examining postcranial elements. The features used for comparison have been chosen in such a way as to maximise the content of the data that are related to biomechanical function. Studies of several anatomical regions—shoulder, pelvis, ankle, foot, elbow, wrist and hand are now available for extant non-human primate species, for man and for australopithecines.

Structure and function in living primates

It is thus information about function rather than genetic propinquity that is presumably best presented by such quantitative studies of skeletal fragments. For many of the details of skeletal form are associated with stresses present as a result of the underlying functions of the bones in the life styles of the different animals. Unfortunately we do not know precisely how to assess for this purpose the many different activities that make a life style. It would seem unlikely for instance that posture would be as important as locomotion because the stresses involved, except in specific parts such as tension-bearing ligaments or compression-bearing callosities, would generally be rather

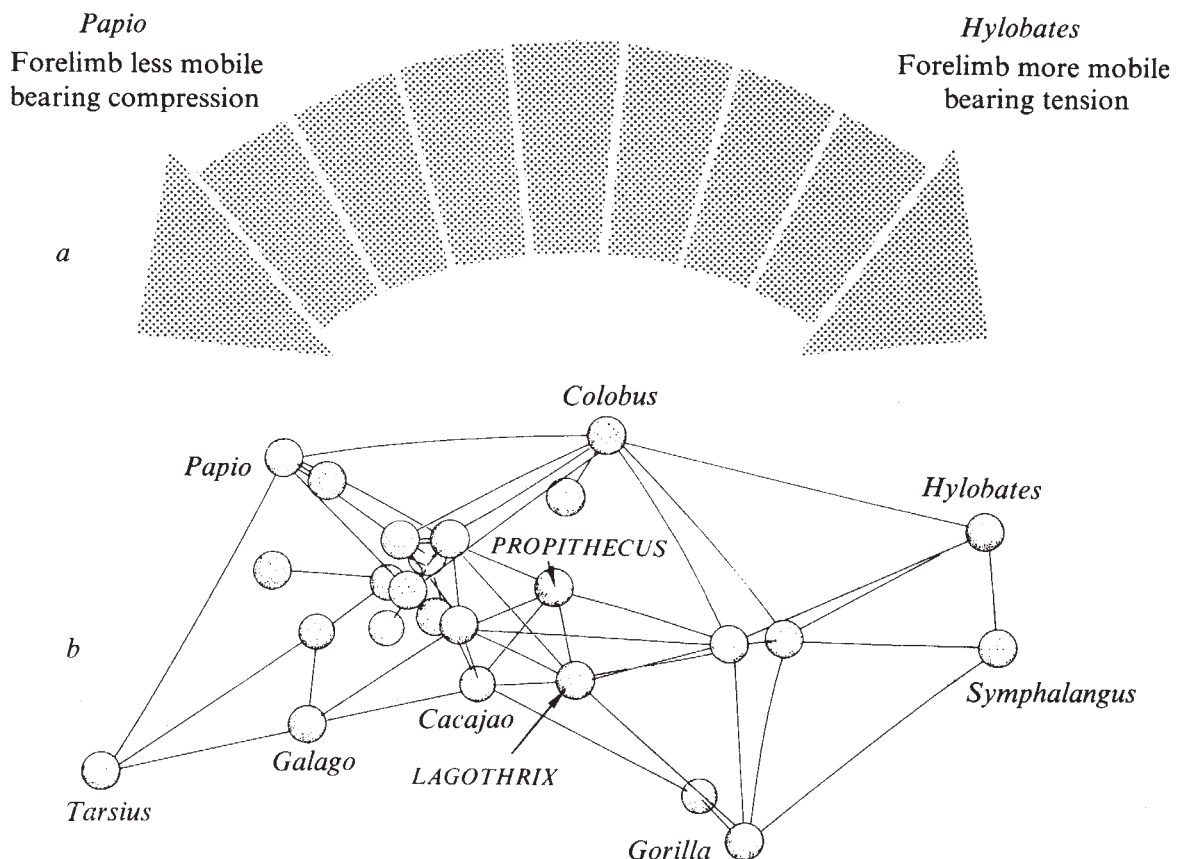
small. It is considerably more difficult to assess the relative importance of functions due to rare but life-saving activities and those associated with ordinary locomotion. And very little is known of the impact of small but unremitting cyclical functions, such as heart beat and respiratory rhythm. Nevertheless, it is to a complex "resultant" of these functions that the shape and structure of individual bones seems to be most related. And because it produces the largest forces locomotion will be a major contributor. This seems to be so whether we study the scapula or the pelvis, the forelimb or the hindlimb, the hand or the foot, although it seems that some areas do provide such functional information rather more readily than do others (E. H. Ashton, R. M. Flinn, C.E.O. and T. Spence, unpublished).

It is thus not easy to characterise structurally important aspects of behaviour. For many years^{20,21} as well as recently^{22,23} this characterisation has been attempted by making locomotor classifications based on the total behaviour of the animals. These classifications have resulted in controversy²⁴ because it is scarcely possible to group the entire activities of the different primates other than to say that they are all different.

When, however, one wishes to draw associations with the structures of particular anatomical regions there is some value in attempting to classify the functions (very broadly conceived) associated with particular anatomical regions. Our early classifications for the forelimb, hindlimb and vertebral column of primates²⁵ proved useful by suggesting aspects of morphology that might be closely related to such aspects of behaviour in widely different species, but is proving less valuable as morphological comparisons are attempted among more similar forms²⁶⁻²⁸.

Accordingly we have now tried to characterise structurally associated aspects of behaviour as spectra of the

Fig. 1 The band-like spectrum of function in the primate forelimb (a) and the model of forelimb structure that can be constructed from a multivariate statistical study of proportions of the forelimb. The model (b) is some thirty standard deviation units in its largest dimension. Based on original data from Professor A. H. Schultz. These results are confirmed by studies of other anatomical regions¹⁸.



functions associated with the appropriate anatomical regions. The location of different animals within such spectra are not constant from anatomical region to anatomical region because the functional relationships of different regions are not the same in all species. The form of the functional spectra for different anatomical regions may vary from rather simple two-dimensional analogies to quite complex multi-dimensional star-shaped models. The use of such complex yet broad, diffuse and relatively nonspecific characterisations is forced upon us in the absence of any detailed understanding of the relationship between bone structure and motor behaviour.

The structural patterns found in each anatomical region in the different animals can be characterised in more detail because of the use of quantitative data from the elements of individual animals and because of the use of multivariate statistical and other methods for adding together many different aspects of form for each animal. On these bases we can be much more confident of the existence of continuous structural spectra, though the accidents of evolution may sometimes apparently leave us with "holes" in our spectra.

Once functional and structural spectra are constructed it may be possible to map from the one to the other. The associations may sometimes provide information about the nature of the causal link between function and structure; at other times they may enable us to understand how functions may have evolved even when we have no temporal data but must rely on parallels for the inferences.

How in practice have such ideas worked? Morphometric studies in primates of the form of the scapula, the arm, the forearm, and the overall proportions of the forelimb as a whole, provide, in their generality, a picture of forelimb structure as a rather simple broad band or swathe-like spectrum ranging from species such as baboons and patas monkeys at one extreme to forms such as gibbons and orang-utans at the other. This seems to correlate fairly well with the notion of a broad band or swathe of forelimb function. At one extreme of such a functional spectrum are species (for example, baboons) in which the forelimb tends to be used in quadrupedal locomotion on a two-dimensional substrate (the ground or the large branches of trees) so that the limb typically moves in a two-dimensional antero-posterior arc and bears mainly compressive forces. At the other end of this spectrum are animals (for example, gibbons) in which the limb tends to be used in a raised position, moving in all three dimensions and bearing tensile forces during a rather wider range of movements in the trees where locomotor, postural, foraging and feeding activities are likely to result in such forces. Most primates are intermediate between the two (Fig. 1).

When, however we look towards morphological studies of the pelvis, of the foot and of the overall proportions of the hindlimb as a whole, we find that the distribution of the non-human primates is different from that revealed in the forelimb studies. On the basis of structure they seem to fall into a star-shaped distribution. Functional considerations in the lower limb in these animals place all those species which may be said to be generally quadrupedal towards the nucleus of the star, whereas those primates that are thought to be more highly specialised are those aligned along different rays of the star. The finding of most interest is, of course, that the particular species placed in the nucleus or in any particular arm of the star do not appear to be especially closely related taxonomically; rather do they seem to have similar structural specialisations for functional elements of locomotion that impinge on the hindlimb (Fig. 2).

With a background of such structural-functional associations for several different anatomical areas in living forms, it should be possible to make functional inferences for fossil

species. Before we attempt such a procedure, however, let us examine some extant forms in this way. We can then compare the result of the method with the behavioural reality in a way that can never be attempted with fossils.

Two species that may be chosen are the woolly monkey (*Lagothrix*) and sifaka (*Propithecus*). In the various analyses of the shoulder, arm, forearm and forelimb as a whole, these two animals are not very distant from one another, both lying in intermediate positions within the broad band-shaped arrangement that stems from each study (Fig. 1). If the functional-structural associations for these studies have some meaning then we would surmise that, whatever the actual behaviour of these animals, they show some parallel in having mobile forelimbs bearing tensile forces intermediate between the extreme species: gibbons and baboons. Study of the behavioural literature confirms that the woolly monkey and sifaka move in different ways but suggests that the parallels in forelimb function are real. Both animals are well known for a considerable degree of three-dimensional movement of the forelimb, for using the forelimbs above the head in tensed or even sometimes in hanging postures, and for using the hands for drawing in terminal branches during feeding, thus producing tensile forces in the limb. Neither species does these things as much or as well as gibbons, as rarely or as poorly as baboons.

In the various analyses of hindlimb structures (of pelvis, and hindlimb dimensions as a whole), however, these two animals, the woolly monkey and the sifaka, are remarkably distantly placed from one another. Within the star-shaped spectrum that results from each of the hindlimb studies neither species lies in the main nucleus of quadrupedal forms; each lies out on a ray. Each is found in association with other species that have similar biomechanical arrangements in the hindlimb. The woolly monkey is close to gibbons and siamangs which do not leap. The sifaka lies with other indriids that leap a great deal, together with one or two other species (*Haplemur*, *Lemur catta*) which leap in a similar manner but not as much (Fig. 2).

On the basis of these species therefore, the inference that might be drawn about their positions in both of the functional spectra from their actual loci in the associated morphological spectra is confirmed from what is actually known of their general activities. But the total behaviour and the overall morphology of each living form is unique unto itself. Thus if these total behaviours and overall morphologies were not known for the woolly monkey and the sifaka—if they were fossils—they could still be described through similarities and differences to other living forms. Most species are, thus, describable as mosaics of the parts of other species. Though there is little point in characterising living species as structural mosaics in this way it is a useful exercise in assessing fragments of fossil species. For although it is unlikely that any particular fossil moved in exactly the same way as any single living species, consideration of the fossil as a mosaic of structures may provide some notion as to what it may have done.

Place of man among non-human primates

In all of the studies presented above, the discussion centres around the non-human primates. Thus, before passing to the examination of fossils, it is of interest to view the position of man. Previous understanding of primate taxonomy and evolution through comparative anatomy suggests that man falls relatively close to the non-human primates in a locus related to the chimpanzee and gorilla (although it is true that traditional taxonomy places the African great apes with the Asiatic great ape, as a little separate from man²⁹). In more recent years data relating to the anatomy of molecules also confirm the overall relationship of man

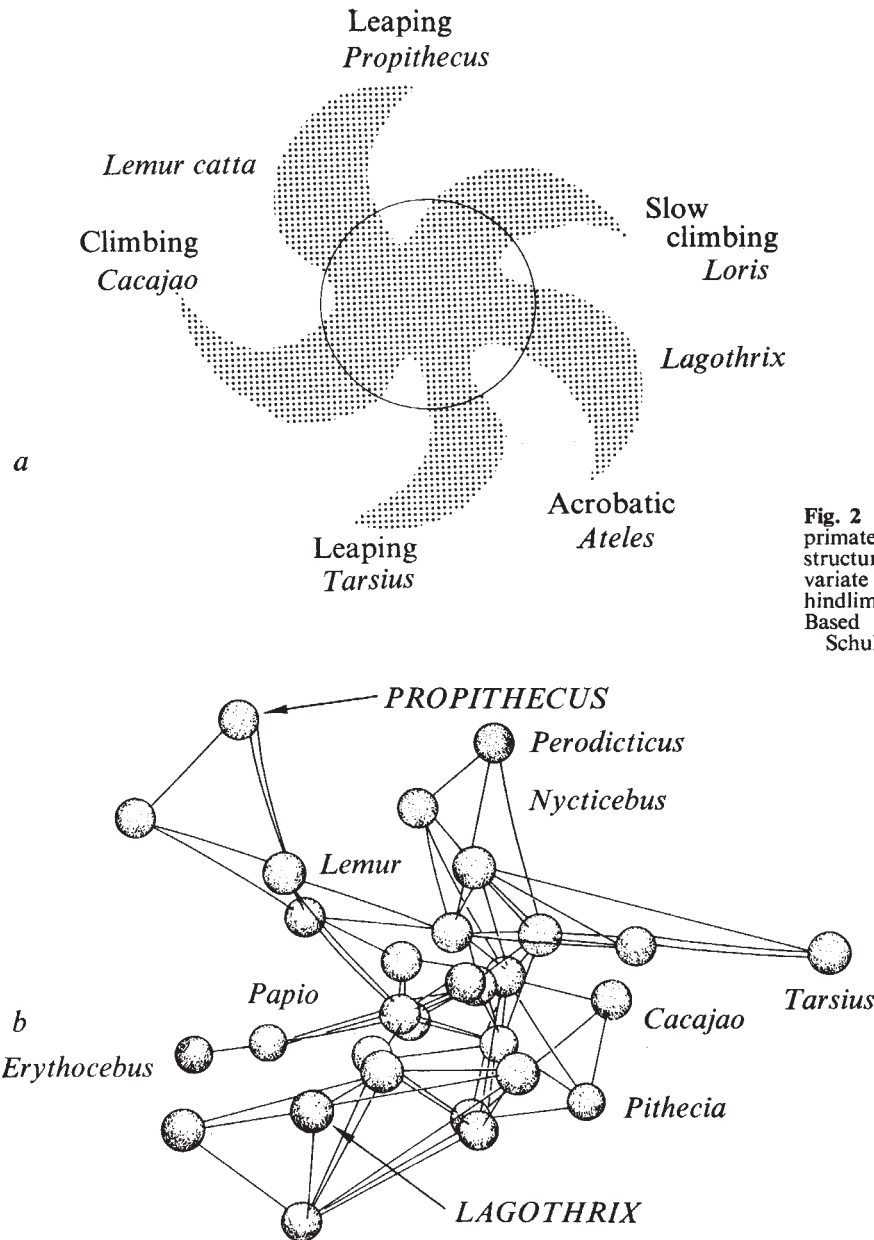


Fig. 2 The star-like spectrum of function in the primate hindlimb (a) and the model (b) of hindlimb structure that can be constructed from a multivariate statistical study of proportions of the hindlimb. The model is some 24 s.d. in radius. Based on original data from Professor A. H. Schultz and confirmed by other studies¹⁸.

to the non-human primates (although it is of interest that, in the details of these more recent studies, the chimpanzee and gorilla are placed unequivocally closer to man with the Asiatic orang-utan somewhat more distant³⁰).

The results of the current studies, in giving more information about structurally relevant aspects of behaviour than about genetic propinquity, do not present these classical pictures; man is not, in these studies, especially close to the primates as a whole or to the chimpanzee in particular. Nor, however, can man be described as a mosaic of other forms. In almost all studies man lies quite separately from the spectra of non-human species although he is, of course, somewhat closer to particular species (for example, sifaka, saki monkey, orang-utan, chimpanzee) than to most others (Fig. 3).

This uniqueness for man does not contradict the findings from traditional morphology or from the molecular studies referenced above. This uniqueness presumably arises from his having a totally different behavioural repertoire from any other primates, and therefore unique functions in different anatomical regions. The lack of a mosaic description presumably results because functional parallels are not available from other living primates. Although all primates can use their forelimbs for manipulation, communication,

feeding, and so on, the overall functions of the human forelimb are different from those of other primates because man does not use this member for locomotion. Although almost all primates can walk on two legs, the functions of the human hindlimb are uniquely different because only man does this habitually and also, and perhaps especially, because only man is utterly unable to use the hindlimb in quadrupedal locomotion.

The findings for man provide, therefore, not only additional important information necessary for studying fossils that may be thought to be close to human, but also further confirmation of the functional, more than any other biological, such as genetic, emphasis to these particular morphological results.

The functional status of australopithecines

The third and final set of comparisons contains the ones in which we have most interest: those between the various fossil genera that are generally believed to be associated with the human lineage.

Fossils such as Neanderthals need scarcely be considered. A great deal is known about their anatomy and we may assume that they are very similar to modern man; surely,

for instance, they were bipeds. It turns out that the only postcranial anatomical region for which we have fragments that have been compared by multivariate morphometrics is a particular ankle bone—the talus. These studies show unequivocally that several different Neanderthals (Spy, Skhül^{10,31,32} and Kiik-Koba^{31,32}) are similar to man and quite different from non-human primates.

A much earlier series of fossils, the talus bones of Miocene apes, have also been studied in this way. These are reminiscent more of gibbons and siamangs than of other living primates. Their next closest morphological similarities are with orang-utans^{31,32}. Thus these particular tali may have belonged to the feet of small, relatively arboreal, at least somewhat acrobatic, seldom-leaping creatures. They are certainly not like those of man, of present-day African apes or Old World monkeys. These fragments do not offer any particular controversy although it would be of great interest if access to the relatively full skeletal remains of *Oreopithecus* could be obtained so that many different anatomical areas could be examined.

Between the very early Miocene apes and ancient man is the tantalising set of fossils known as *Australopithecus*. Although most workers feel that the overall position of these fossils is adequately fixed, with a taxonomic label as clearly Hominidae, an evolutionary label as on the line to man or very close to it, and a functional label as a human type of biped and tool user and maker, the postcranial fragments available have not been fully studied. Most conclusions so far mirror the conventional ideas obtained from skulls and teeth. But our current studies are providing rather different ideas.

In the multivariate investigations reported here, the various australopithecine fossils are usually quite different from both man and the African apes (except in those features which are common to all hominoids or to all anthropoids). The fossils can be described by mosaic characteristics (Table 1). Viewed as a genus they are a mosaic of features unique to themselves and features bearing some resemblances to those of the orang-utan. As with the results for the woolly monkey and sifaka this mosaic

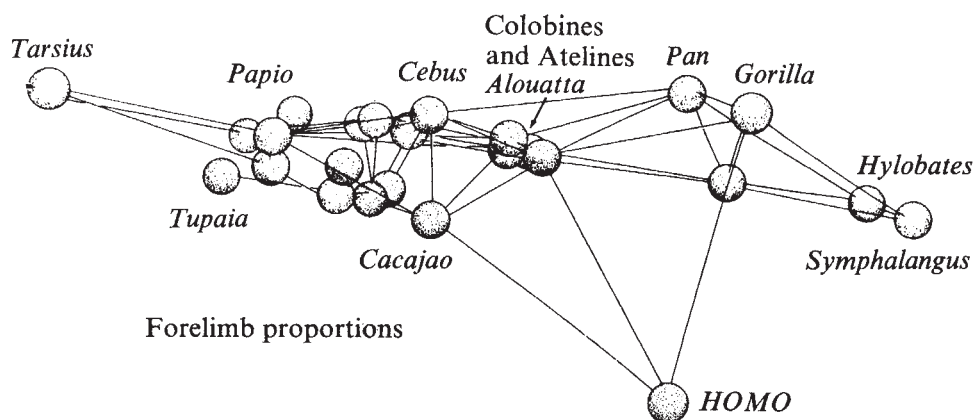


Fig. 3 The models of the structure and function of the fore- and hindlimbs rotated to show the position of man¹⁸.

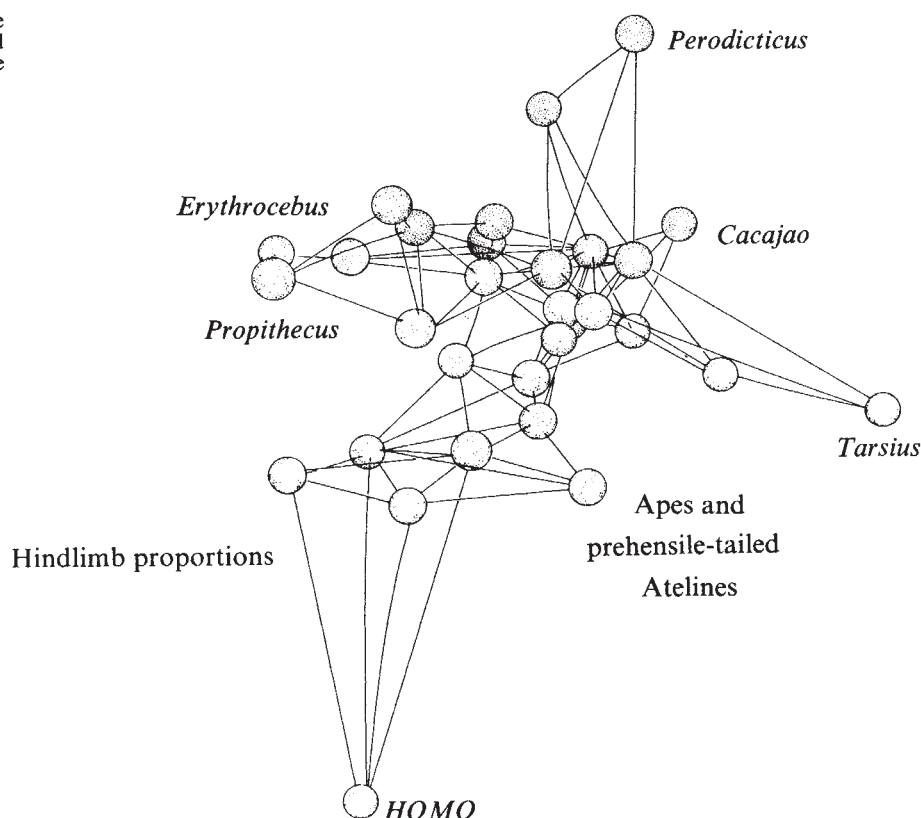


Table 1 Summary of results of multivariate studies of australopithecine fragments

Generally recognised resemblances with man	Resemblances in current studies	
	Orang-utan	Unique
Scapula	+	
Clavicle	+	
Humerus*		+
Metacarpal*		+
Phalanges*	+	
Pelvis		+
Talus	+	
Toe phalanx		+

*These anatomical areas thought to be somewhat less like man than the remainder.

condition presumably does not speak to genetic relationships. Such resemblances as there are between these fossils and the orang-utan must result from functional parallels. And because similarities with the orang-utan are only in some anatomical regions and not in others, because the overall comparison is mosaic in nature, it is clear that the actual overall mode of locomotion of the orang-utan today is not the model for these creatures. If the fossils have ankle, hand and shoulder bones patterned somewhat after those of the orang-utan then we can only surmise that perhaps, as the orang-utan, the fossils had ankles, hands and shoulders adapted for climbing. Because they have pelves that have articular relationships parallel to those of man, we may guess that perhaps they stood and moved upright with a vertical load distribution. But because the muscular features of the pelvis are positioned in a way more like those of the great apes, we must estimate that their muscular arrangements were therefore rather similar to those associated with climbing and perhaps quadrupedalism. They have relatively small articular surfaces in the hindlimb as compared with the forelimb, which parallels most closely the orang-utan, and contrasts markedly with man who has big articular surfaces in the leg compared with the arm. They may have been bipedal in a way that is no longer seen, but have retained abilities for climbing, and perhaps minor arboreal acrobatics such as might be found in an intermediately sized ape-like creature. If these estimates are true, then the possibility that any of the australopithecines is a direct part of human ancestry recedes; the study of their possible posture and locomotion becomes a fascinating adventure in its own right^{17,18}.

Incidentally it must be pointed out that this mosaic result for the australopithecines is not an artefact of grouping the various australopithecine specimens of possibly different species into a single genus. First, the difference between the species, (if they truly exist) is considerably less than between the species in many extant genera. Second, in those cases where we have postcranial specimens of more than one australopithecine there is not usually found any great difference. The talus bones from Olduvai (*A. africanus* or "*H. habilis*") and from Kromdraai (*A. robustus*) are scarcely distinguishable from one another and fit in well with a whole host of non-human Pleistocene tali from Africa³². The humeral fragments, though distinguishable from each other, are quite different from present-day man or any living apes. The various femoral remains of both robust and gracile forms show the small heads and inclined femoral necks that might be expected in animals capable of quadrupedal activities.

Independent supporting evidence

What is the possibility that this radical re-interpretation of the postcranial elements of these fossils may be correct? Three other pieces of evidence support these ideas and are independent of those assembled in our laboratory.

One comes from the recent finding at East Rudolf by Richard Leakey³³ of a new talus lying between layers that have been dated at between 1.5 and 2.6 Myr by argon isotope techniques. This specimen is thus as old as, if not appreciably older, than the australopithecine talus from Olduvai noted earlier in this article. Yet this new specimen seems to be much more like that of man as far as can be judged from the published picture and descriptions. Further description and examination using multivariate statistical methods³⁴ confirms that it is indeed similar to modern man and unlike the australopithecine specimens. The second indication comes from the finding, again by Leakey³⁵, of a skull dated at almost 3 Myr (certainly more than 2 Myr) and with an endocranial volume of nearly 800 cm³ and possibly more (compare with *Homo erectus* at 900 cm³); this may be contrasted with the australopithecines, the most recent of which may be less than three-quarters of a million years old and the cranial capacities of which are often only about 450 cm³, smaller than of a large gorilla disregarding difference due to overall bodily size. The third piece of evidence comes from a find made some years ago—a fragment of arm bone perhaps four or more million years old from Kanapoi. This has already been shown to be very similar to that of modern man³⁶, and some of our demonstrations clearly support that contention¹⁸.

Thus, unless tali, crania and humeri evolved through cycles such that they were more like man at early stages, less like him at later australopithecine stages, and more like him again at an even later human stage, then the australopithecines must have lain on an evolutionary side branch for a long time.

These pieces of evidence may also mean that perhaps as long as five million years ago or even longer there may have been creatures living that were generally somewhat similar to *Homo erectus* and therefore classifiable as man in a way that we must now deny to *Australopithecus* (whether named "*H. habilis*", "*H. africanus*" or whatever else). The removal of the different members of this small-brained curious genus, *Australopithecus*, into several parallel side lines distinct from the direct line to modern man would make it easier to accept the findings described here. And this re-assessment is to be seen in the revision by at least one authority³⁷ of the age of the Taung australopithecine, so that it postdates the arrival of true *Homo* in Southern Africa according to the conventional notion. At the same time the possible age of a human-non-human link is rapidly being increased; on one assessment, which originally dated a chimpanzee-human common ancestor at around 3 Myr³⁸, a revised estimate gives a date of six or even 10 Myr³⁹ while many other authorities^{40,42} maintain, of course, that much longer periods characterised this particular evolutionary divergence.

The existence of individuals somewhat like *Homo* (in the very few features available for comparison) this much earlier has further, broader implications. The length of time available for the psycho-social evolution of *Homo* may well have been almost an order of magnitude greater than had been believed. Knowing as we do the enormous speed of psycho-social evolution by comparison with biological evolution, then perhaps more of modern man's character should be attributed to psycho-social, and less to biological, evolution than some would have us suppose.

Thus the cruel and sophisticated expression of man's aggression towards his own species may be less dependent on a previous animal base and more dependent on the relatively longer span of psycho-social evolutionary time. The extreme intricacies of human culture may likewise reflect considerably less on a previous basic behaviour shared with non-human primates and somewhat more on psycho-social evolution. Human intellectual prowess may have had as much as ten times more time to increase from

a level comparable to that of some non-human stage of behaviour than we have previously realised.

More evidence of earlier forms that are more like man than australopithecines will be found even though these latter must also have existed at earlier periods in time. Presumably more fossil remnants will be discovered outside Africa because over such a long time period, human or prehuman populations must have existed in other places, with migrations, and with multiple evolutionary lines.

We may have to accept that human bipedality is far older than previously guessed. With that may go a far more ancient free forelimb with its associated abilities for tool using and tool making. We may have to accept that the australopithecine form (or forms) of locomotion, tool using and tool making may be merely one (or more) unsuccessful evolutionary experiments existing in parallel with those of man. We may well have to accept that it is rather unlikely that any of the australopithecines, including "*Homo habilis*" and "*Homo africanus*" can have had any direct phylogenetic link with the genus *Homo* except perhaps at earlier times.

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articles

Age of KBS Tuff in Koobi Fora Formation, East Rudolf, Kenya

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Conventional K–Ar dates on pumice from the KBS Tuff horizon in the Koobi Fora Formation at East Rudolf, Kenya, distinguish two tuff units. Samples from area 10 and area 105 give an age of 1.60 ± 0.05 Myr whereas those from area 131 give 1.82 ± 0.04 Myr. Both ages differ from the previously published age of 2.61 ± 0.26 Myr obtained by $^{40}\text{Ar}/^{39}\text{Ar}$ technique.

PLIO-PLEISTOCENE lacustrine and fluvial strata exposed along the east side of Lake Rudolf, Kenya, now known as 'East Rudolf', have been under study since 1969 when Behrensmeyer began geological investigations, which led to the identification of the KBS tuff in area 105 (Fig. 1)^{1–4}. These strata, resting unconformably on late Miocene and Pliocene volcanics, are notable for their extraordinarily rich assemblages of fossil vertebrates, which include a large number of hominid remains. Artefacts also occur in some places^{5–8}.

Interspersed in the stratigraphic sequence are several rhyolitic tuffs, mostly reworked, which have served as marker beds and for calibration of the fossil vertebrates and hominid artefacts by potassium–argon dating^{9–11} and in one instance by fission-track dating¹². Dates on tuffs within the Koobi Fora Formation range from 3.18 Myr for the Tulu Bor Tuff in the Lower Member, to 1.22 Myr for the Chari Tuff and the correlated Karari Tuff in the upper part of the Formation. The KBS Tuff from the type locality in area 105 was assigned an age of 2.61 Myr, although subsequent age determinations resulted in a broad scatter of dates, both older and younger than this value^{10,11}. Contamination by ancient bed rock material during the reworking of the tuffs was suggested to account for the anomalously old dates, whereas subsequent alteration, 'overprinting', of the pumice fragments used for dating, by alkaline-rich and possibly heated ground water may explain the anomalously young dates by partial loss of radiogenic argon.

Using the incremental heating technique of $^{40}\text{Ar}/^{39}\text{Ar}$ dating, Fitch and Miller believed they could resolve these overprinting events by analysis and interpretation of age spectra, and

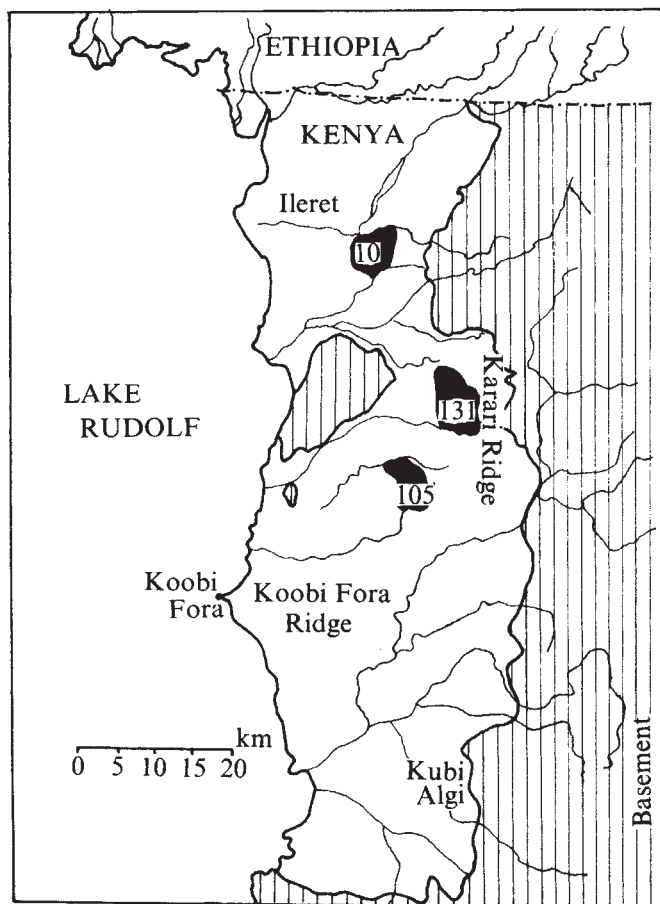


Fig. 1 Map of the East Rudolf region, showing the sampling areas.

arrived at the 'best apparent age' for the KBS Tuff of 2.61 ± 0.26 Myr (ref. 10).

When some palaeontologists compared fauna associated with the KBS Tuff in East Rudolf with those of other, supposedly well calibrated localities, the reliability of the date of 2.61 Myr for the KBS was questioned. Although Maglio found that the morphology of elephant fossils fit with a 2.5 Myr date⁷, Cooke and Maglio, in 1972, pointed out that fossil pigs from below the KBS Tuff horizon at East Rudolf seemed to correlate best with those from beds dating close to 2 Myr in the Omo River area to the north in Ethiopia¹³⁻¹⁶. Because of the great interest in the dating and the question of reliability raised by the scatter of results from the KBS Tuff, we repeated the dating using the conventional method of K-Ar dating.

It is argued that the conventional K-Ar method is unable to evaluate results obtained by the $^{40}\text{Ar}/^{39}\text{Ar}$ method, because it assumes that all non-radiogenic argon in the sample has the same relative abundance as in the atmosphere, an assumption demonstrably wrong in some cases. Although this may be true for a single determination, multiple K-Ar determinations made on several samples of a tuff, and on more than one type of material can be plotted on an isochron diagram to show the isotopic composition of the non-radiogenic argon in the tuff.

With respect to the $^{40}\text{Ar}/^{39}\text{Ar}$ dating method, Dalrymple and Lanphere have pointed out (personal communication) that even after 5 yr of research with this method they occasionally obtain anomalous ages for geologically young rocks (late Tertiary and younger) which they are unable to explain. Furthermore, the interpretation of the $^{40}\text{Ar}/^{39}\text{Ar}$ isochrons that yield multiple apparent ages from a single phase, such as Fitch and Miller have obtained on KBS sanidines, is uncertain. Their interpretations rely on oversimplified and unproved models for the diffusion of argon in solids, both in nature and in the

laboratory procedure necessary to make an age determination. For this reason we feel that their results, even when they are reproducible to high precision, may be an artefact of experimental procedure, and thus not geologically meaningful.

Analytical procedure

During the summer of 1974 samples of pumice were collected from outcrops now classified as the KBS Tuff in three localities. One additional pumice sample from the eastern part of area 105 had been collected by Lucas previously for geochemical analysis during palaeomagnetic sampling in the summer of 1973. These samples were subsequently prepared for dating, using selected pumice fragments which were first examined both by eye and in thin section. Most sections showed the principal minerals, sodic sanidine and pyroxene, to be fresh, although the enclosing vesicular glass ranged from highly altered in most specimens to fresh in only three. Calcite filled most vesicles, although zeolite was present in some.

Sanidine samples were separated from the glass with bromoform after the pumice had been cleaned, crushed and sieved. The concentrates were then treated with 1 N HCl to remove calcite, and 5 % HF for 1 min to remove adhering clay. Splits of these samples were then analysed for argon, using

Fig. 2 Isochrons for KBS Tuff. a, $^{40}\text{Ar}/^{36}\text{Ar}$ against $^{40}\text{K}/^{36}\text{Ar}$; b, ^{40}Ar against %K. ●, Data from area 131; ▲, areas 105 and 10. Plots obtained by least-squares fitting. The ratio of $^{40}\text{Ar}/^{36}\text{Ar}$ in air is 296.0.

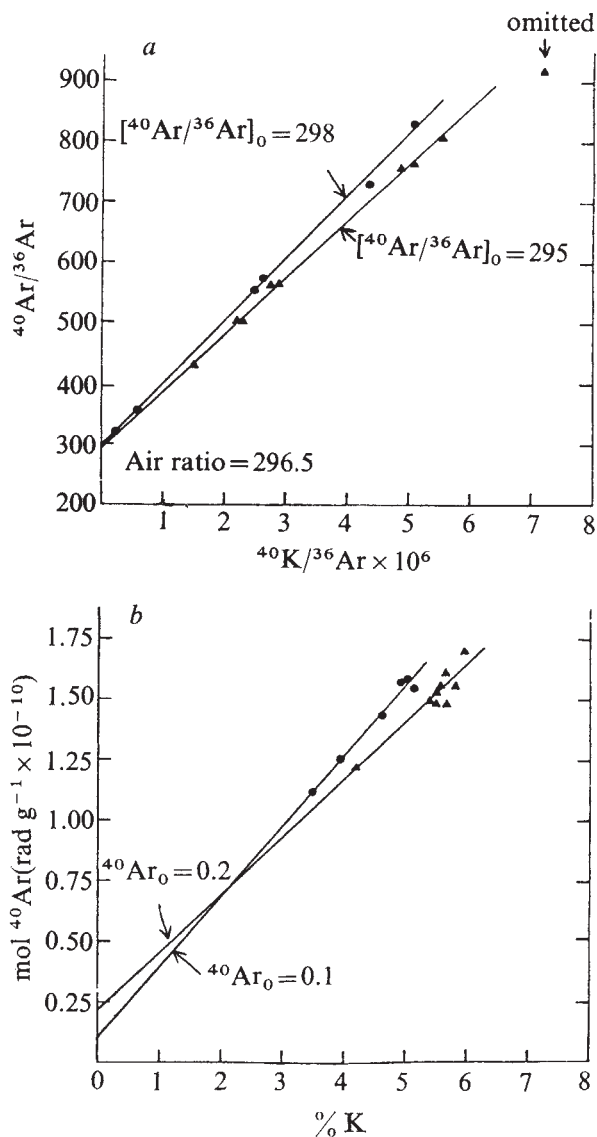


Table 1 KBS Tuff ages (Myr)

Area 105	Area 10	Area 131
1.65 (S) } 0001 1.50 (S) }	1.54 (S) } 0002 1.60 (S) }	1.84 (G) } 1.83 (S) } 0001 1.81 (S) }
1.62 (S) } 1.56 (S) } 0002 1.62 (S) }	Mean of 2 = 1.57	1.83 (G) } 1.85 (S) } 0002 1.73 (S) }
1.68 (G) } Lucas 1.61 (S) }		Mean ($\pm$ s.d.) of 6 = 1.82 ± 0.04 Myr
Mean of 7 = 1.61 Mean ($\pm$ s.d.) of 9 from 105 and 10 = 1.60 ± 0.05 Myr		

G, glass; S, sanidine.

Samples with detrital contamination have been omitted.

procedures described by Dalrymple and Lanphere¹⁷, and for potassium on a Zeiss PF5 flame photometer following dissolution of samples in HF and neutralisation.

The first dates (KA 2816, KA 2817, KA 2818, and later KA 2851) obtained from samples prepared in the manner described showed no agreement (see Table 3). Microscopic examination of the remaining concentrated crystals revealed numerous yellow grains of rounded and altered K-spar, clearly detrital in origin and probably derived from ancient bedrock. These grains had been tightly cemented on the convoluted surfaces of the rounded pumice, and had escaped detection in spite of careful cleaning. Subsequent work proved that it was virtually impossible to exclude these detrital sand grains completely when mineral separations were made using heavy liquids. To avoid this problem, we carefully crushed individual pumices in a hand mortar, extracting the large phenocrysts with tweezers under a magnifying glass or binocular microscope. The phenocrysts were then treated with HF, crushed again, and put through a magnetic separator to remove included and adhering pyroxene grains.

Only three specimens 131-0001, 131-0002, and the Lucas sample from area 105 had glass fresh enough for both sanidine and glass age determinations to test for possible effects of overprinting. Since sanidines and glass have markedly different

physical and chemical properties, it is highly unlikely that any substantial overprinting event would produce an exactly proportional loss of argon, such that their apparent ages would remain the same. It is equally unlikely that several sanidines from widely separated samples would yield identical apparent ages, had overprinting occurred, as the intensity and duration of these events are not everywhere uniform. Thus, when concordant dates are obtained on two different phases from the same sample which also closely agree with single dates on other samples the probability becomes increasingly large that these dates are an accurate estimate of the true age of their formation.

Results

Three different pumice clasts from the KBS Tuff at area 105 and one from area 10 (Ileret) give an average age of 1.60 ± 0.05 Myr for nine analyses, while two separate pumice clasts from the tuff in area 131 (Karari Ridge), hitherto correlated with the type KBS in area 105, give an average age of 1.82 ± 0.04 Myr on six analyses (Table 1).

The narrow range of dates and high precision obtained on all samples, once sufficient care was taken in sample preparation,

Table 2 Concentrations of niobium, zirconium, yttrium and rubidium in pumice

Locality	Sample number	Concentrations (p.p.m.)			
		Nb	Zr	Y	Rb
KBS 105	ER72-105-0004	500	757	144	98
	Lucus-TC-A	502	746	144	101
	Lucus-TC-B	503	735	139	103
KBS 131	ER74-131-0001	333	1,357	121	87
	ER74-131-0002	926	1,664	303	146

Nb and Y were analysed using BCR-1 as standard. Zr and Rb were analysed using G-2 as standard.

we believe distinguish the tuff in area 131 from the KBS in area 105 and area 10. This same distinction is seen by the comparison of average K contents for the uncontaminated sanidine separates used for age determination. The sanidines from area 105 and area 10 average 5.6% with a range of 5.36–5.94%, whereas those from area 131 average 4.90% with a range of 4.60–5.11%.

Table 3 KBS analytical data

KA no.	Sample no.	Material dated	%K	%atmospheric ⁴⁰ Ar	mol ⁴⁰ Ar rad g ⁻¹ × 10 ⁻¹¹	Age × 10 ⁶ yr	Remarks
2816	ER74-010-0002	Sanidine	5.167 ± 0.013	17.8	6.203	6.90 ± 0.05	detrital contamination
2817	ER74-105-0002	Sanidine	5.035	37.8	1.755	2.01 ± 0.03	detrital contamination
2818	ER74-105-0002	Sanidine	5.006	41.2	2.089	2.40 ± 0.03	detrital contamination
2823	ER74-131-0001	Sanidine	4.808	40.6	1.807	2.16 ± 0.03	detrital contamination
2836	ER74-131-0001	Sanidine	4.993 ± 0.019	35.7	1.584	1.83 ± 0.02	hand picked crystals, HF 2 min
2837	ER74-131-0001	Glass	3.922 ± 0.020	92.5	1.252	1.84 ± 0.07	heavy liquid separation, HF 2 min
2841	ER74-131-0002	Sanidine	4.896 ± 0.031	51.6	1.569	1.85 ± 0.03	hand picked crystals, HF 2 min
2842	ER74-131-0002	Sanidine	5.110 ± 0.045	40.6	1.539	1.73 ± 0.03	hand picked crystals, coarser fraction than KA 2841
2843	ER74-131-0002	Glass	3.484 ± 0.0002	82.9	1.105	1.83 ± 0.03	heavy liquid separation, HF 2 min
2850	ER74-105-0001	Sanidine	5.665 ± 0.040	32.2	1.479	1.50 ± 0.02	hand picked crystals, HF 2 min
2851	ER74-105-0001	Sanidine	~5.94	31.0	2.605	2.53 ± 0.02	detrital contamination
2851R	ER74-105-0001	Sanidine	5.936 ± 0.007	39.1	1.698	1.65 ± 0.02	same as KA 2851 with detrital grains picked out by hand
2852	ER74-010-0002	Sanidine	5.785 ± 0.004	68.9	1.545	1.54 ± 0.02	hand picked crystals, HF 2 min
2854	Lucas-105-East	Glass	4.171 ± 0.0001	52.7	1.217	1.68 ± 0.01	heavy liquid separation HF 10 min to remove zeolite
2855	ER74-105-0002	Sanidine	5.468 ± 0.024	58.9	1.481	1.56 ± 0.02	hand picked crystals, HF 5 min
2856	Lucas-105-East	Sanidine	5.487 ± 0.020	36.7	1.529	1.61 ± 0.02	hand picked crystals, HF 5 min
2857	ER74-105-0001	Sanidine	5.543 ± 0.021	52.5	1.558	1.62 ± 0.02	hand picked crystals, HF 5 min
2859	ER74-105-0002	Sanidine	5.643 ± 0.034	58.7	1.613	1.65 ± 0.02	hand picked crystals, HF 5 min
2861	ER74-010-0002	Sanidine	5.359 ± 0.0009	38.8	1.485	1.60 ± 0.01	hand picked crystals, HF 5 min
2869	ER74-131-0001	Sanidine	4.598 ± 0.0002	53.4	1.441	1.81 ± 0.02	hand picked crystals, HF 5 min

$$^{40}\text{K}/\text{K} = 1.18 \times 10^{-4}; ^{40}\text{K}_\lambda = 5.480 \times 10^{-10} \text{ yr}^{-1}; ^{40}\text{K}_{\lambda\beta} = 4.905 \times 10^{-10} \text{ yr}^{-1}; ^{40}\text{K}_{\lambda\epsilon} = 0.575 \times 10^{-10} \text{ yr}^{-1}.$$

X-ray fluorescence analyses¹⁸ show the distinctive Nb, Zr, Y, and Rb contents of pumice glass from the two tuff units (Table 2). Sample 0001 from area 131 and those from area 105 are, however, similar in appearance, being composed of dark brown glass with open vesicles enclosing large euhedral sanidine phenocrysts, whereas sample 0002 from area 131 is composed of a grey glass with elongated tubular vesicles enclosing less abundant sanidine phenocrysts. These pumices with fresh glass are rare compared with those which are completely altered to clay minerals and zeolites.

Although the difference between KBS-105 and KBS-131 is clear, the data for the samples from area 131 show that two different pumices have been sampled. Even though they are of the same age, they may be derived from different eruptive centres. This introduces the possibility that older pumices may also be present in the KBS tuff horizon which could account for the 2.61 Myr date reported by Fitch and Miller. For this reason, all dates obtained on pumices should be considered as maximum ages for the tuff itself.

The high precision and concordancy of dates obtained on all samples, in spite of the fact they ranged from fresh to highly altered, indicates to us that none has lost significant argon after deposition.

This conclusion is further supported by two isochron plots (Fig. 2) (and see also ref. 19). The $^{40}\text{Ar}/^{36}\text{Ar}$ against $^{40}\text{K}/^{36}\text{Ar}$ isochron shows that the non-radiogenic argon component in both age groups is nearly the assumed atmospheric composition.

The ^{40}Ar against % K isochron indicates a slight excess ^{40}Ar component for each group, which would make the apparent ages too old. The narrow range of potassium in this isochron, however, makes the error in extrapolation large; but the fact

that a positive rather than negative ^{40}Ar intercept results, argues against argon loss for either sample group.

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Statistical mechanics and topology of polymer chains

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The statistical-mechanical treatment of closed polymer chains based on algebraic topology is proposed. Using the Monte-Carlo method numerical results were obtained for the probability of knot formation during random closing of polymer chains of different length. For very rigid chains such as DNA double helix the probability of knot formation is rather great. Topological restrictions in a system of two polymer chains are shown to lead to a specific topological interaction between them.

THE detailed statistical-mechanical theory of polymer chains^{1–4} may be rigorously applied only in the case of linear, unclosed polymer chains. Interest has been shown in the particular case of closed polymer molecules, connected on the one hand, with successful synthesis of a new type of chemical compound, catenanes, consisting of two topologically linked polymer molecules⁵. On the other hand, closed circular DNA molecules are widely distributed in nature. DNA may be found in linear as well as in closed circular form at different stages within the cell^{6,7}. In some cases, two or more DNA molecules form catenanes.

One would expect the statistical-mechanical properties of closed polymer molecules to differ considerably from those

of linear molecules. This difference originates from the fact that in the former case a topological state formed during closing of the chain must remain unchanged in the course of any conformational transformations of the molecule. This problem is closely related to that of computing the probability of formation of a given topological state by a polymer chain while closing.

Different topological aspects of polymer statistical mechanics have been discussed^{8–16}, but a solution to the problems of statistical mechanics of closed polymer chains has not been worked out. The major difficulty arises in the formulation of an algorithm to distinguish the different topological states of the chain. In other words, one has to be able to reduce a given entangled polymer coil formed by a closed chain to its simplest form for which the subsequent disentanglement is not possible (Fig. 1). Crippen¹⁵ has attempted to solve this problem in a more direct way, by developing an algorithm which progressively decreases the number of self-intersections of the chain on its projection. Crippen's algorithm has, however, proved ineffective. Edwards¹⁰ has proposed an integral invariant of a knot but this invariant has been shown¹⁷ to be incorrect.

We have proposed^{17,18} the use of the results of algebraic topology, in which highly effective invariants of knots and links were developed, and the generation of closed chains on a lattice by the Monte-Carlo method. One may then calculate statistical-mechanical properties of isolated chains of different

length, and of a system of two closed polymer chains. We present here the results of such calculations. Details of the theory of knots and links are given in refs 19–21.

Types of knots and links

Knots are classified according to the minimum number of intersections on their projections (Fig. 2 shows knots with less than six intersections). A complete table of all 84 knots having less than ten intersections is given in ref. 21.

A topological invariant best suited to our purposes is the Alexander polynomial, and the values, $\Delta(t)$, corresponding to simplest knots, are given in Fig. 2. For an unknotted closed chain (trivial knot), $\Delta(t) = 1$.

Note that any two chains which are equivalent topologically have been shown to have the same Alexander polynomial²⁰. Computation of this value thus solves in principle the problem of reduction of a given arbitrary entangled polymer coil to its simplest form (Fig. 1).

We have simplified computation of the polynomial to a computer algorithm¹⁷. Note that some degeneracy exists for

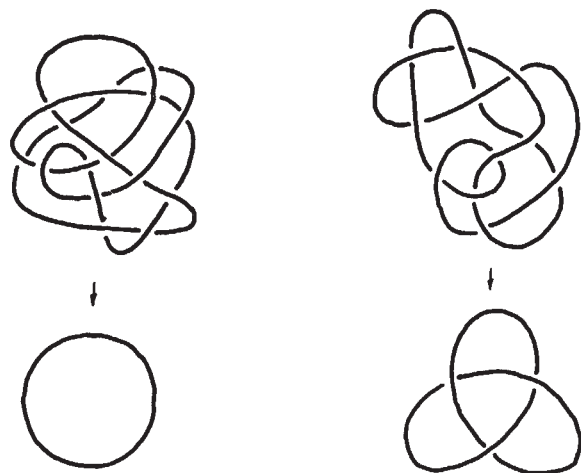


Fig. 1

the invariant under consideration, that is, two topologically different knots may have the same Alexander polynomial. Such coincidences, however, are extremely rare. Specifically the simplest non-trivial knot having $\Delta(t) = 1$ contains eleven intersections on its projection (for example, ref. 17). In the framework of this paper such degeneracy may be considered negligible.

Links may be classified in much the same way as knots (for example, Fig. 3 shows four of the simplest links). The Alexander polynomial in this case, represents a function of two variables $\Delta(s, t)$. Examples of this polynomial are shown in Fig. 3. For two closed chains not linked with each other $\Delta(s, t) = 0$.

Methods of calculation

To calculate the statistical-mechanical properties of closed polymer chains the Monte-Carlo method was used. The polymer chains were simulated by the paths of random self-avoiding walks on a three-dimensional body-centred lattice. To obtain only closed chains an effective algorithm¹⁷ was used. Note if self-intersections of the chain are excluded in Monte-Carlo calculations, one must calculate the statistical weight for each chain when computing the probabilities of different states of the chain^{17, 22, 23}.

Calculations have been made for two models of a polymer chain. The first model corresponds to a 'thick' or flexible

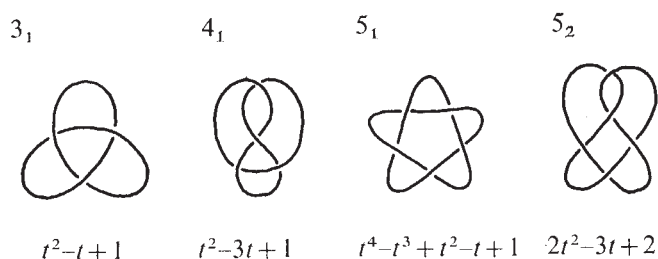


Fig. 2 Simplest non-trivial knots and their Alexander polynomials.

polymer chain for which the Kuhn segment length is close to its thickness. Such a chain has been generated directly by self-avoiding random walks on the lattice. The second model corresponds to a 'thin' or very rigid chain for which the Kuhn segment length is much greater than its thickness. To obtain such a chain we allowed the end of the generating path to reach a previously occupied node of the lattice. But each time such an intersection occurred the lattice as a whole was shifted along a randomly chosen direction for a very small distance compared with the period of the lattice. The random walks were continued on this new displaced lattice. Such chain generation guarantees the loss of a true self-intersection, at the same time enabling any two segments far apart on the chain to pass very close to each other in space. By contrast, in the case of the 'thick' polymer chain the most distant segments are not permitted to approach one another closer than the (approximate) period of the lattice.

Using one of the methods outlined above a closed non-self-intersecting chain was generated, and its corresponding statistical weight calculated. Each chain was classified as to its specific topological type by calculating its Alexander polynomial. The probability of formation of a particular topological state as a result of random closing of a chain was computed as a sum of statistical weights of chains belonging to a given topological type divided by the total sum of statistical weights of all generated chains.

When considering topological interaction of two closed chains only the model of the extremely 'thin' chain was considered. First, two chains of the same length were generated independently in this case, and were brought closer together, the distance between the centres of mass of the chains having a value R . This was achieved by simple parallel displacement without any deformation of the chain; during such displacement the chains were permitted to penetrate one another freely. When displacement was finished the system formed was classified into its specific topological state by calculating its Alexander polynomial. The probability of formation of a particular topological state was computed as above for the case of a single chain.

Probability of knot formation

What is the fraction of chains of a given length L (number of Kuhn segments) expected to form a trivial knot or a knot of particular type (see Fig. 2) after random closing? Our calculations have shown that this probability depends on the flexibility

Fig. 3 Simplest linkages of two chains and their Alexander polynomials.

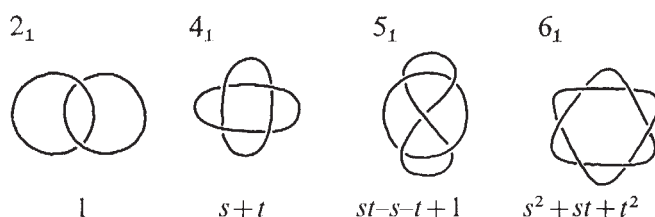


Table 1 Probability of knot formation in the model of thick (or flexible) chain

Chain length (L)	No. of chains inspected	No. of knotted chains	Probability of knot formation
50	49,000	55	$(0.5 \pm 0.2) \times 10^{-4}$
100	13,000	98	$(1.6 \pm 0.8) \times 10^{-4}$

of the polymer chain. For very flexible (or thick) chains for which the segment thickness is close to its length the probability of knot formation is extremely small even for chains containing as many as 100 segments (Table 1). In calculating the probability of knot formation for the model of thick chains, taking into account the correct statistical weights of different paths is important. This may be attributed to the greater compactness of a knotted chain compared with an unknotted one; as a result, the former has, on average, a much smaller statistical weight. Thus, a simple division of the number of knotted chains by the total number of generated chains leads to an incorrect (overestimated) value of the probability of knot formation (Table 1).

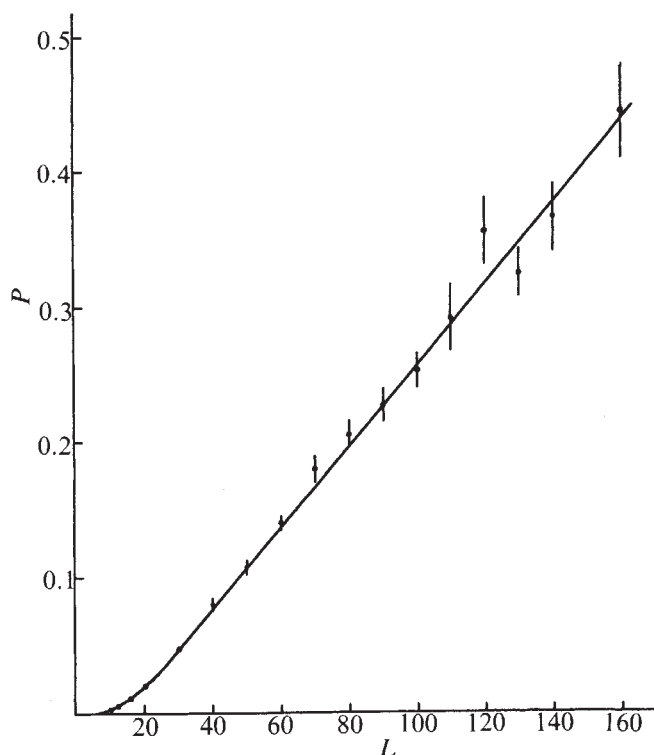
In contrast, the probability of knot formation for the model of a rigid (or thin) chain having segment length much greater than its thickness is quite appreciable. Figure 4 shows the calculated dependence of the probability of formation of a non-trivial knot during random closing of a thin polymer chain on its length (in terms of number of segments), L . A chain containing 150 segments has to form a knot in approximately 40% of all cases. Obviously, different knots are formed with different probabilities (Table 2). If short chains mainly form simple knots (trefoil, type 3_1) the fraction of trefoils decreases with increase in chain length, and for $L = 150$ such chains amount to only 40% of the total number of knotted chains.

Topological interaction between two polymer chains

Topological restrictions arising in the system of two closed polymer chains lead to a specific topological interaction between them. Let us consider two ideal (infinitely thin) polymer chains having no excluded volume effects. This means that the single effect of interaction between chains, and between different segments of the same chain, consists of the elimination of passages of one segment through another. If both chains are unclosed, a state of one chain will not depend on the state of another; thus, there will be no interaction between them. (We are considering only equilibrium properties of polymer chains. Elimination of passages of one segment through another in unclosed chains should lead to some kinetic effects, and these may be considerable for sufficiently long chains. Such kinetic problems, however, are beyond the scope of our paper.) The closing of one of the chains will not change the situation. But as soon as we close both chains the situation changes drastically. Indeed, the closing of both chains converts the system to some particular topological state (the linkage of some type, Fig. 3) which has to remain unchanged in the course of any subsequent deformations of the chains. This topological restriction decreases the conformational entropy of the system. As a result, free energy of the system of two closed polymer chains depends on its topological state and on the distance between the chains.

The number of conformations of a pair of chains belonging to a specific topological state is the total number of conformations of two isolated closed chains multiplied by the probability of formation of a given topological state during random closing of two chains.

We have restricted ourselves to the simplest case of statistical-mechanical properties of two unlinked closed polymer chains. We need to know therefore only the probability P_0 of formation

**Fig. 4** Probability of non-trivial knot formation during random closing of thin polymer chain involving L segments.

of unlinked state during random closing of two chains as a function of the distance R between their centres of mass.

An unexpected feature of the results of Monte-Carlo calculations of P_0 for four chains length (chain length in each pair assumed to be equal), (Fig. 5) was that all follow equation (1), within the limits of calculation errors

$$P_0 = 1 - A_0 \exp(-\alpha_0 R^3) \quad (1)$$

where A_0 and α_0 are functions of chain length L (Table 3). But the dependence of P_0 on R is the same inside the entire region of lengths studied. The dependence of the free energy of the system, which can be treated as the 'potential' for interaction between chains, on interchain distance is given by the following equation

$$F_0 = -kT \ln P_0 \quad (2)$$

In summary, the topological restrictions arising in the system of two closed unlinked polymer chains lead to a repulsive potential of interaction between them, having an entropic origin (equations (1) and (2)).

Such interaction between chains must lead to the non-ideal behaviour of a gas or solution containing closed polymer molecules. In the case of the equation for the state of such a

Table 2 Fraction (%) of knots of different types formed during a random closing of a thin chain

Chain length L	3_1	4_1	Type of knot 5_1	5_2	All others
10	98	2	0	0	0
20	83	10	1	3	3
40	76	12	5	3	4
60	66	13	4	6	11
80	58	13	6	6	17
100	58	14	3	6	19
120	58	9	2	6	25
140	53	8	2	9	28
160	46	10	5	8	31

gas (or solution), the second term of virial expansion must arise

$$p = ckT(1 + cB), \quad (3)$$

where c is the number of polymer molecules in the unit volume of gas (or solution), and B is the second virial coefficient. This value is determined by the interaction between a pair of molecules and may be calculated from the potential for interaction between them (for example, ref. 24). In our case, for the potential determined by equations (1) and (2) the calculations lead to

$$B = (2/3)\pi(A_0/\alpha_0) \quad (4)$$

Values of B calculated from equation (4) for four values of L are given in Table 3. How large has the effect of mutual repulsion of closed polymer chains originating from topological interaction between them proved to be? To answer this question we have compared values of B with those for a hypothetical gas of rigid impenetrable spheres having dimensions corresponding to the mean-square dimensions of polymer coils under consideration. A mean-square radius of gyration of the closed polymer chain was chosen as the radius of a corresponding rigid sphere². Values of B^* for such a gas of equivalent rigid

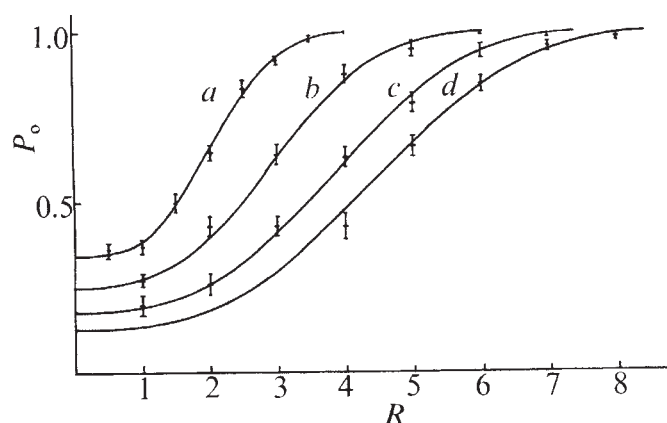


Fig. 5 Dependence of probability of formation of unlinked state by two polymer chains as a result of random closing on the distance R between their centres of mass. Different curves correspond to different values of chain length L ; a, 20; b, 40; c, 60; d, 80. Values of R and L are expressed in terms of the number of segments. Points are results of machine calculations. Curves are drawn from equation (1) for values of A_0 and α_0 presented in Table 3.

spheres are given in Table 3. At small L , B is smaller than B^* but these values become very close as chain length increases, indicating that the effect of topological interaction between closed unlinked polymer chains effectively forbids any mutual penetration of the chains. Note that this effect originates only from topological restrictions and takes place at the Flory theta point in the absence of any common excluded-volume effects.

Discussion

Theoretical consideration of statistical-mechanical properties of closed polymer chains encounters great difficulties because the topological state of the chains has to be taken into account. It is possible to overcome such difficulties using results obtained in algebraic topology, on the one hand, and the Monto-Carlo method on the other. As a result the calculations of statistical-mechanical properties of chains involving as many as 150 segments have been carried out.

Our calculations relate to an unsolved problem in the search for a closed molecule which forms a knot^{5,9,16}. Our results show that for flexible chains, to which the common hydrocarbon polymers belong, the probability of knot formation during their random closing is extremely low (Table 1). Moreover, this probability increases slowly with chain length and for chains containing as many as a hundred segments it is as small as $\approx 10^{-4}$. The search for knotted molecules obtained as a result of random closing of flexible polymer chains does not show much promise. Much more promising is the method of random closing in the case of rigid chains. Double-helical DNA molecules fall into this category, because their segment length exceeds the width by a factor of 50. Some DNA molecules such as DNA from bacteriophage λ are very promising in the search for knotted molecules, as in linear form they have cohesive ends and may be readily closed *in vitro*^{6,7}. Our calculations predict that about 40% of the λ DNA molecules which contain about 150 segments should be knotted after closing *in vitro*. Knotted molecules may be easily confused with unknotted ones during electron microscopic inspection of a preparation; in the search for knotted molecules special precautions are needed. Such a search in preparations of closed λ DNA *in vitro* have not been undertaken.

Knotted DNA cannot finish the replication cycle because two daughter molecules will be linked with each other. Thus, the great probability of knot formation even for such comparatively short molecules as λ DNA predicted by our calculations, indicates that inside the cell a special mechanism must be found which forbids the formation of a knotted chain during DNA closing *in vivo*. To rule out this possibility completely requires the location of the DNA molecule on a two-dimensional surface. The cell membrane is best suited to this purpose. Data have led to the hypothesis about the location of λ DNA inside the cell on a membrane (see ref. 6, p. 100). Naturally, other more specific mechanisms forbidding knot formation inside the cell may be assumed.

The topological interaction between two closed polymer chains may considerably affect the dimensions of a DNA molecule inside the helix-coil transition interval because melted regions represent such closed polymer chains. Such closed chains must be practically impenetrable, one for another, thus simulating large excluded-volume effects. Such anomalous excluded-volume effects have been observed for partially melted DNA by Shugali *et al.*¹². They have shown that dependence of DNA specific viscosity on temperature may be understood only if a complete absence of mutual penetration of

Table 3 Parameters of the potential for interaction between unlinked closed polymer chains (α_0 , A_0) and values of B for different values of chain length, L

L	α_0	A_0	B	B^*
20	8.2×10^{-2}	0.66	17	36
40	2.6×10^{-2}	0.75	60	101
60	1.2×10^{-2}	0.82	143	185
80	0.8×10^{-2}	0.87	228	286

coiled regions is assumed. They¹² have also postulated that topological restrictions play an important role in this effect. Our results make such interpretation probable. Note, however, that in real experimental conditions a contribution from the common excluded-volume effect as well as from kinetic effects may be considerable. Thus, strictly speaking, the purely topological effects may be demonstrated only if the lack of mutual penetration of closed chains and free penetration of linear chains would occur in the same conditions. In addition to a quantitative estimation of the value of topological interaction our results enable an estimation of the probability of formation of linked molecules (catenanes) during random

closing of polymer chains in solution. The concentration of catenanes c_k may be calculated from:

$$c_k = c^2 B \quad (5)$$

where c is the concentration of closing molecules. Note that equation (4) and Table 3 give the value of B expressed in units of l^3 , where l is segment length. Thus, to obtain an absolute value of B the value obtained using equation (4) and listed in Table 3 should be multiplied by l^3 . In addition, equation (5) is derived using the assumption that the chains have not interacted before closing and that $cB \ll 1$. Our results lead to conclusions about the probability of catenane formation, which are in accord with previous predictions⁹. We know of only one attempt to obtain data on the probability of catenane formation²⁵.

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Heat resistance of bacterial endospores and concept of an expanded osmoregulatory cortex

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The extreme resistance of bacterial endospores to heat may result from dehydration of the central protoplast brought about and maintained by osmotic activity of expanded electronegative peptidoglycan polymer, and positively charged counterions associated with it, in the surrounding cortex. The cortex may thus act as a specialised osmoregulatory organelle. Changes in the environment which would be expected reversibly to affect osmotic properties alter the heat resistance of spores.

ALTHOUGH many of the enzymes and other macromolecular components of bacterial endospores are as heat-sensitive as those of vegetative forms when extracted from disrupted cells, the resistance of the intact spores exceeds that of the corresponding vegetative forms by factors as great as 10^5 -fold¹. The calcium dipicolinate complex has been widely quoted¹ as being important in heat resistance, but considerable doubts have been raised by the isolation of mutants which produce dipicolinic acid-free spores and which are nevertheless still heat resistant². Lewis *et al.*³ suggested that such extreme resistance might result partly from dehydration of the central protoplast within the spore by compressive contraction of the surrounding cortex. We¹ reasoned that based on the known properties of peptidoglycan, of which the cortex is largely composed⁴, its mass, and the volume occupied by this structure in spores, the cortex was more likely to be expanded than contracted, a possibility pointed out by Alderton and Snell⁵. Peptidoglycan in spores is electronegative and loosely cross linked^{6,7} and could therefore be expanded by electrostatic repulsion of adjacent negatively charged groups in the polymer. Expansion would be expected in the absence of high concentrations of cations, while contraction or

collapse would be expected in the presence of high concentrations of cations, and particularly in the presence of multivalent ones. Such cation-induced contraction of isolated vegetative cell wall peptidoglycan has been described^{8,9}.

We¹⁰ therefore tested the expanded cortex hypothesis by treating spores in ways that would be expected to cause collapse of the expanded structure, and then measuring the heat resistance of the treated spores. The predicted heat sensitising occurred in the presence of high concentrations of multivalent cations as long as the spores had been treated in some way to increase permeability of the outer spore coat, for example, by the use of coat-defective mutants, or by chemical modification of the coat with combinations of urea with thiol compounds, or by the use of a divalent cation ionophore to increase the cation-permeability of the spore.

Table 1 Heat sensitivity of variously treated spores of *Bacillus cereus* in calcium chloride

Treatment*	Survivors (%) after heating† in Water	Calcium chloride
Untreated control	85	98
Urea	105	83
Mercaptoethanol	105	88
Urea + ethanol	83	93
Urea + mercaptoethanol	83	0.23
Urea + thioglycolic acid	78	0.35
Urea + dithiothreitol	101	5.2

B. cereus T spores were grown on potato glucose yeast extract agar at 30 °C and washed and cleaned as described in ref. 10.

*Spores (about 10^8 per ml) were incubated with reagents at 37 °C for 1 h then washed by centrifuging in water. Reagent concentrations were: urea, 7.2 M; β-mercaptoethanol, 10% (v/v); thioglycolic acid, 1% (v/v); dithiothreitol, 6.5% (v/v); ethanol, 10% (v/v). Reagents used at pH 3 and at pH 7 gave essentially similar results. † Treated, washed spores were heated in water or CaCl₂ solution (4 M) at 70 °C for 30 min.

This article reviews the evidence and presents new data that support the expanded cortex hypothesis and which also indicate a new possibility: that the cortex functions to maintain the enclosed spore protoplast in a relatively dehydrated state not solely by expansive pressure, but also by osmosis. Osmoregulation by the cortex can be influenced experimentally with predictable effects on the heat resistance of the spore.

Heat sensitisation of spores with cations

Tables 1 and 2 summarise data showing that: (1) mutant spores and spores treated with thiol compounds in urea, so that the coats become permeable even to molecules as large as lysozyme¹¹⁻¹³, became heat sensitive in the presence of high concentrations of salts of multivalent cations; (2) in contrast, normal untreated spores of various species, and spores treated with various control reagents that did not contain thiol compounds, remained heat resistant in high concentrations of calcium chloride; (3) effectiveness of the multivalent cations increased in the order Sr^{2+} , Ca^{2+} , Mg^{2+} , La^{3+} ; (4) low concentrations of multivalent cations (that is less than molar CaCl_2) were relatively ineffective; (5) monovalent cations (for example, Na^+ , K^+) did not cause heat sensitisation at high concentrations, equimolar with the multivalent cations; (6) thiocyanate and guanidinium ions, which are among the most effective ions known to decrease the stability of proteins to heat¹⁴, did not decrease the heat resistance of the treated spores. The extent of heat sensitisation by multivalent cations was great; that is generally well over 1,000-fold after heating for 10 min at 70 °C, although survivor curves were nonlinear (Fig. 1). In separate experiments the heat sensitivity of urea-mercaptoethanol-treated spores in calcium chloride was reversed if the salt was removed, for example, by centrifuging and resuspending the spores in water before heating¹⁰.

That the heat-inactivated spores were truly non-viable, rather than simply made dormant through inactivation of their germination mechanism, was suggested by the failure of techniques such as washing the spores in chelating agents (sodium dipicolinate, sodium ethylene diamine tetraacetate) and incubation in calcium dipicolinate agar¹⁵ or agar containing lysozyme¹⁶ to induce their recovery (Table 3); such techniques often recover viable but heat-damaged spores. The observations summarised in Tables 1 and 2 are compatible with the hypothesis that the heat resistance of spores is normally maintained when the cortex peptidoglycan is electrostatically expanded and that heat resistance is reduced when the peptidoglycan is electrostatically cross linked and collapsed; for example, sufficient concentration of multivalent cations is allowed to penetrate the permeable coats of mutant or thiol-treated spores. There is consider-

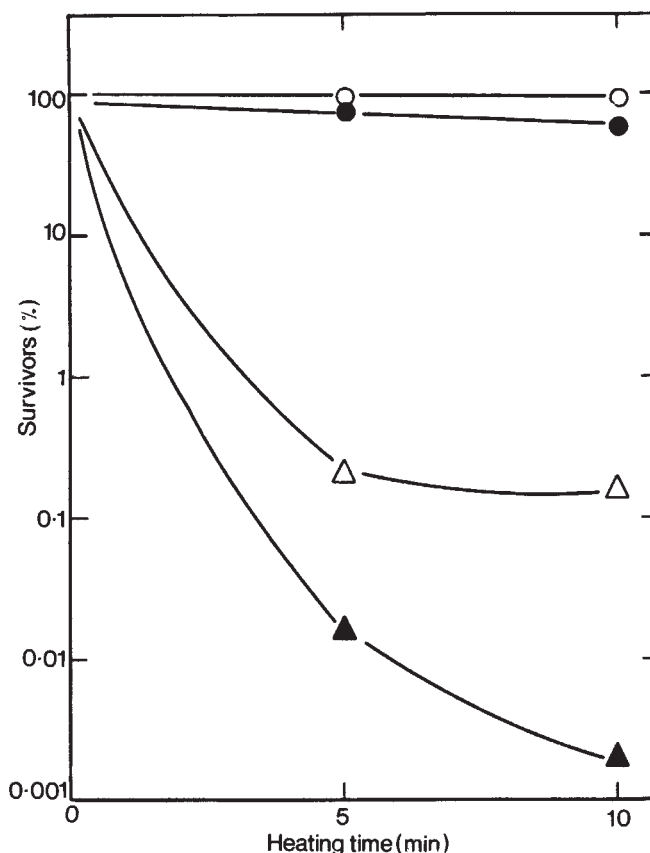


Fig. 1 Heat sensitivity of spores of *Bacillus megaterium* NCTC 7581 in CaCl_2 . Spores were treated with urea + mercaptoethanol as indicated in Table 2 then washed and heated at 70 °C in CaCl_2 solutions at the concentrations indicated in the figure. ○, water; ●, 2 M CaCl_2 ; △, 3 M CaCl_2 ; ▲, 4 M CaCl_2 .

able evidence that the central protoplast in spores is relatively dehydrated¹⁷. The expanded cortex hypothesis supposes that expansive pressure in the cortex brings about this dehydration mechanically. Lewis *et al.*³ pointed out that a pressure of about 50 atm would be sufficient, for example, to compressively dehydrate wool keratin to 15% water.

Alternatively, the cortex could bring about dehydration of the enclosed protoplast more plausibly by other means. Since it contains a loosely cross linked polymer with an excess of free carboxyl groups over amino groups^{6,7}, the cortex will normally contain a sufficient concentration of free positively charged counterions to maintain electrical neutrality: the polymer plus counterions together will be

Table 2 Effect of salts on heat sensitivity of coat-modified spores

Organism*	Treatment†	Survivors (%) after heating‡ in										
		Water	NaCl (4 M)	KCl (4 M)	SrCl ₂ (4 M)	CaCl ₂ 4 M	3 M	2 M	1 M	MgCl ₂ (4 M)	La(NO ₃) ₃ (4 M)	KNCS (5 M)
<i>B. cereus</i> T	Untreated control	85	89			98						
<i>B. cereus</i> T	Urea + mercaptoethanol	100	90	64	1.1	0.85	7.8	58	77	0.1	0.01	100
<i>B. stearothermophilus</i>	Urea + mercaptoethanol	90				0.47	1.6	24	49			100
<i>B. megaterium</i> NCTC 7581	Urea + mercaptoethanol	100				0.0012	0.08	30	55			
<i>Cl. sporogenes</i> NCIB 2111	Urea + mercaptoethanol	100				1.8						
<i>Cl. perfringens</i> 8.6 (coat-defective ¹²)	Untreated	98	97			0.1		2.9				
<i>B. megaterium</i> ATCC 9885 (coat- defective ¹³)	Untreated	100				15			90			

*Spores were produced and cleaned as in ref. 10.

†Conditions of treatment as in Table 1 except incubation was at 60 °C for 1 h.

‡Heating conditions as in Table 1 except for *Cl. perfringens* (100 °C, 10 min) and *B. stearothermophilus* (100 °C, 30 min).

NaCl and CaCl_2 solutions adjusted to pH 3 and pH 7 with HCl or NaOH gave similar results to those shown.

Table 3 Attempted recovery of spores of *B. cereus* T heat-inactivated in calcium chloride

Treatment*	Survivors (%)† enumerated in media		
	Control	Lysozyme	CaDPA
	agar	agar	agar
Control	0.43	0.56	0.54
NaEDTA	0.38	0.40	0.42
NaDPA	0.44	0.48	0.54
Urea + mercaptoethanol		1.06	

*Spores were first treated with urea + mercaptoethanol (60 °C, 30 min) then inactivated by heating in CaCl₂ (4 M, 70 °C, 30 min). Survivors were either plated directly (control) or washed with sodium ethylene diamine tetraacetate (NaEDTA, 100 mM, pH 7), or sodium dipicolinate (NaDPA, 100 mM, pH 7) or reincubated with urea + mercaptoethanol (60 °C, 30 min) to ensure maximal lysozyme sensitivity before plating.

†Medium was plate count agar (PCA, Oxoid). Lysozyme was incorporated in PCA at 100 µg ml⁻¹ and 20 mM CaDPA (ref. 15), a concentration which is germinative for this organism.

osmotically active. For example, assuming a 30% polymer gel of high molecular weight (for example, 20,000) in the cortex, with three carboxyl groups per 1,000 daltons, the osmotic pressure exerted could be in excess of 30 atm. Such an osmotic pressure would certainly dehydrate the enclosed protoplast provided that the protoplast did not contain high levels of low molecular weight soluble components, since it is the molarity that largely determines the osmotic pressure. (In fact, the major low molecular weight components in spores are few—principally dipicolinic acid, glutamic acid and other acidic components¹⁸ which, with the high levels of calcium that are present, will be mostly insoluble at physiological pH values.) For example, a solution of the single pure protein haemoglobin is at equilibrium with a solution at 30 atm at a water content of only about 30% (ref. 19). If proteins and other components in the spore protoplast are condensed or associated into multimeric units, their osmotic coefficients will be reduced²⁰ and the water content could well be even below this and closer to the values of 20% or less that have been indicated by refractive index measurements of spores^{21,22}.

Osmotic manipulation of spore heat resistance

If the heat resistance of bacterial spores depends on osmotic dehydration of the protoplast by the surrounding cortex, one can predict that heat resistance should respond to osmotic manipulation. Therefore, to test the hypothesis further, spores of *Bacillus cereus* were first treated with urea plus mercaptoethanol, as in Table 1. These spores were then heated in calcium chloride plus sucrose at a concentration estimated to give an osmotic pressure high enough to maintain dehydration of the spore protoplast. Spores heated in this mixed solution were found to have retained resistance to heat (Table 4). Thus, the observed heat sensitisation in calcium chloride is explicable in terms of electrostatic cross linking of peptidoglycan electronegative groups, loss of counterions causing a decrease in osmotic pressure in the cortex, and consequent rehydration of the enclosed protoplast: re-establishment of heat resistance in sucrose is explicable in terms of dehydration of the rehydrated protoplast, brought about simply by osmosis. A problem remaining is that the calcium chloride itself will exert an osmotic pressure even greater than that exerted by the sucrose and should also cause dehydration, unless it is able to permeate the protoplast.

The level of sucrose needed to maintain the resistance of such sensitised spores of *B. cereus* was found in separate experiments to be above 2 M. Sucrose at 2 M would exert an osmotic pressure of more than 40 atm and one might infer that this value represented a lower limit for the osmotic pressure normally existing within the cortex of spores of this organism. That such protection occurs is not remarkable since protection of vegetative cells from

heat inactivation by dehydration (and plasmolysis) by solutions of non-permeant solutes like sucrose is well known, and the magnitude of the protection can reach several hundredfold²³.

Mechanism of spore heat resistance

Figure 2 summarises the new hypothesis, which has a number of attractive features. It is compatible with the observed high total water content of spores (about 70%) as measured by Black and Gerhardt²⁴ and yet allows for a relatively dehydrated protoplast (in accordance with the high refractive indices of spores^{21,22}), since the cortex region of the spore would be relatively water-filled although in osmotic equilibrium with an enclosed, water-depleted protoplast. Presumably, since water does not continually diffuse into the spore, the system comes into equilibrium as a result of mechanical constraints and pressures developed by the cortex. Osmoregulatory and pressure-imposing functions of the expanded cortex are therefore interdependent. The high apparent specific gravity of spores in equilibrium gradient centrifugation experiments (that is sometimes > 1.4, ref. 25) is readily explained since the low molecular weight solutes used to form such gradients would be expected to permeate the cortex space²⁶, and therefore indicate dry spore rather than wet spore density. The concept of an osmotically active cortex accommodates well the observations that spores held at low equilibrium relative humidities (ERH) show greater water sorption than vegetative cells^{27,28}. The concept is compatible with the increase in resistance that occurs when spores are suspended in certain highly concentrated solutions²⁹, and also with the observations that the resistance, in particular, of relatively heat-sensitive spores, increases greatly with decrease in ERH³⁰, for equilibration at a sufficiently low ERH would lead to loss of water from the cortex, causing an increase in osmotic pressure and consequent further dehydration of the enclosed protoplast as equilibrium was re-established. The hypothesis does not conflict with the observations of Alderton and Snell³¹ that treatment with acid reduces the heat resistance of spores, since such treatment would displace positively charged counterions from the cortex by protonating peptidoglycan carboxyl groups, thus reducing expansion and lowering osmotic pressure. Re-equilibration at high pH values would be expected to reimpose expansion, increase the osmotic pressure exerted by the cortex and reinstate heat resistance, as is found experimentally³¹.

The hypothesis predicts that the outer regions of spores (cortex and coats), being relatively wet, should collapse on drying. Surface collapse was inferred by gas displacement studies³² and by light scattering analyses of single spores³³. That the cortex is normally expansive is also indicated by electron micrographs of disrupted spores in which cortex material seems to expand into the space vacated by the extruded protoplast contents³⁴.

If the cortex is an electronegative expanded polymer, then an expansion mechanism must operate during sporulation. Sporulation specific peptidases have been described that hydrolyse peptidoglycan peptide components³⁵ and may operate to allow controlled expansion. Alternatively,

Table 4 Osmotic reversal of the heat-sensitisation of *B. cereus* T spores

Heating menstruum	Survivors (%) following heating*
Water (control)	100
Sucrose	123
CaCl ₂	0.69
CaCl ₂ + sucrose	84.2

*Spores were incubated with urea + mercaptoethanol as indicated in Table 2 then heated at 70 °C for 30 min in water or in CaCl₂ (3 M) containing no further addition or containing sucrose (3.6 M).

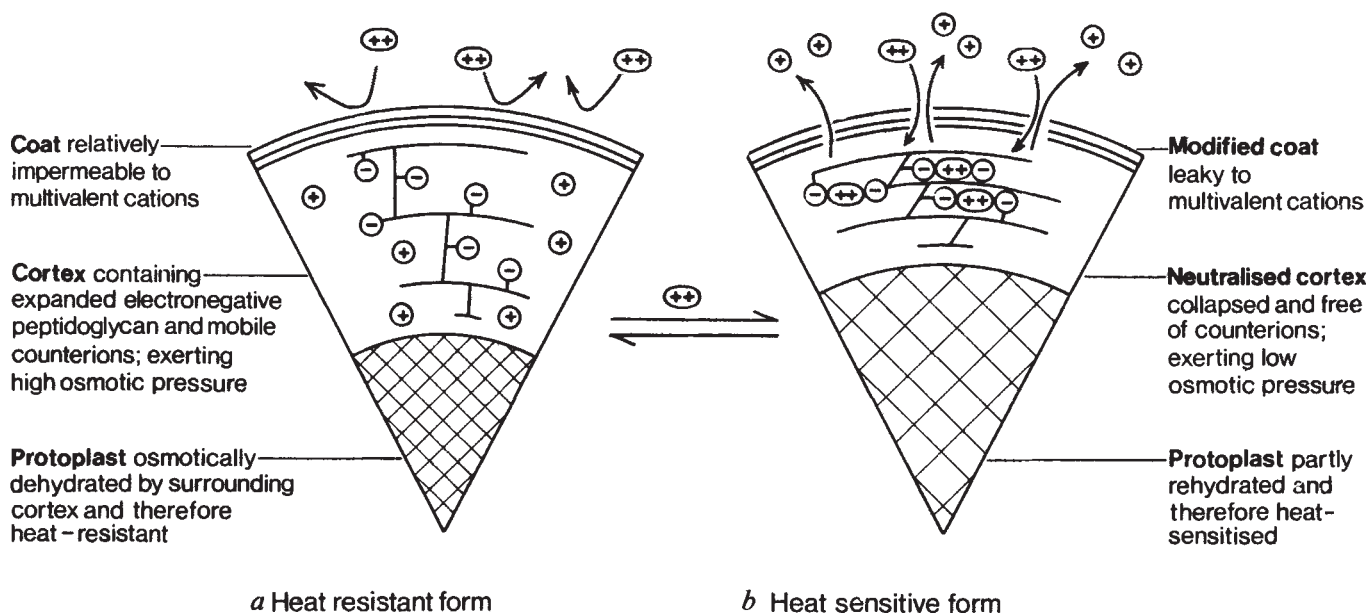


Fig. 2 Diagrammatic representation of the osmoregulatory expanded cortex in normal heat-resistant spores (a) and in coat-modified spores made heat sensitive by multivalent cations (b). —, Negatively charged groups on cortex peptidoglycan, +, monovalent positively charged mobile counterions, ++, multivalent cations.

removal of cross linking cations from this region (for example, by newly synthesised dipicolinic acid) could bring about expansion and the consequent increase in osmotic activity. In its final form, spore peptidoglycan is certainly more electronegative and less cross linked (that is, more expandable) than vegetative polymer⁶, and the relative content of the major electronegative component, diamino-pimelic acid, has been shown to be particularly high in spores of the most heat-resistant species³⁸.

The fact that only spores with modified coats are susceptible to heat sensitisation by multivalent cations suggests that the coats of normal spores have an important cation-protective role. This is open to further study.

The hypothesis has several important implications regarding the dormancy of spores. Calcium dipicolinate, which is present within bacterial spores, will initiate germination if added exogenously³⁷. It is possible that endogenous calcium, which is present at high levels in spores, performs this task during normal germination. Movement of sufficient calcium into the cortex compartment could neutralise electronegative groups, leading to cortex collapse and replacement of counterions. Dring and Gould³⁸ found release of spore-bound K^+ to be the earliest event in germination of spores of *B. subtilis*, occurring at the time when dormancy and heat resistance are rapidly lost³⁹, and it is interesting to speculate that this K^+ might represent counterions displaced from the cortex compartment. The extreme dormancy of dipicolinic acid-negative, low calcium mutant spores¹ may reflect their inability to neutralise the cortex polymer.

Finally, the idea that osmoregulation within a single cell by an organelle like the cortex contributes to resistance, and possibly also to dormancy, may not be peculiar to bacterial endospores. Maintenance of dehydration is typical of resistant and dormant systems in diverse prokaryotes and eukaryotes, and thick-walled structures around resting bodies such as spores, conidia and cysts are common⁴⁰. Some of these contain electronegative polymers, for example, polyuronic acids in *Azotobacter* cysts⁴¹; peptidoglycans in microcysts of *Myxococcus xanthus*⁴² and spores of *Streptomyces*⁴³. Examination of the possible osmoregulatory functions of such structures may prove worthwhile.

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letters to nature

A very high velocity 1,612-MHz OH source near the galactic centre

USING the 25-m radiotelescope at Dwingeloo, we have recently completed the initial stages of a large scale OH survey. The detailed results will be reported later. The survey is designed to yield an unbiased set of data for all four 18-cm lines of OH.

The first part of the survey project was carried out at a frequency of 1,612 MHz with a closed-cycle helium-cooled parametric amplifier and a system noise temperature of 42 K on cold sky. We used a 256-channel autocorrelation spectrometer in a frequency switched mode with a total bandwidth of 2.5 MHz (a total velocity coverage of 465 km s^{-1}) switching over 2.5 MHz. At 18 cm the HPBW of the Dwingeloo telescope is $31'$.

A region of about 12 square degrees around the galactic centre was among the areas observed. It was sampled every 0.3° in both galactic longitude and latitude. Here we discovered a double-peaked absorption feature close to the galactic centre which appeared in several adjacent grid points. Subsequent analysis, however, showed it to be an emission feature in the reference band and therefore at a high velocity.

Confirmatory observations were obtained on August 7, 1975 with the dual-channel 18-cm receiver (system noise temperature of 120 K on the galactic centre) of the 100-m telescope in Effelsberg. The instrument was used in a total power mode with the two halves of the 384-channel autocorrelation spectrometer arranged to receive the two orthogonal polarisations, which were then averaged. Figure 1 shows the final spectrum measured in this manner. The form of the line profile and the velocity separation of the two components (26 km s^{-1}) is typical of that of type IIb OH/IR stars¹. The integrated powers of the two components are approximately equal, that of the lower velocity component being $8.0 \times 10^{-22} \text{ W m}^{-2}$ and that in the higher velocity component being $7.8 \times 10^{-22} \text{ W m}^{-2}$. Both components seem to consist of at least two features with our frequency resolution of 6.5 kHz.

The most striking characteristic of this source, however, is its high mean radial velocity of -341 km s^{-1} . From interpolation across a grid of observed points we derive its position to be

$$\begin{aligned} l'' &= 0.330 \pm 0.005^\circ, \text{ RA}(1950) = 17 \text{ h } 43 \text{ min } 59.0 \pm 1.2 \text{ s} \\ b'' &= -0.193 \pm 0.005^\circ, \text{ dec}(1950) = -28^\circ 44' 13 \pm 18'' \end{aligned}$$

The source seems to be pointlike with the $7.9'$ beam of the 100-m telescope. Its projected distance from the galactic centre is 67 pc (assuming the Sun is at a distance of 10 kpc). A search of this confused region on the Palomar Sky Survey reveals no unambiguous optical counterpart.

High velocities are not uncommon in the direction of the galactic centre². Rougoor and Oort³ have given a value of $\pm 200 \text{ km s}^{-1}$ for the radial velocity range for interstellar material physically close to the centre. The velocity of the source we have found, however, is far outside this range. Comparison with Thackeray's list⁴ of optical high velocity objects shows that the observed velocity is among the highest found in the Galaxy. This may be considered as supporting evidence that the source has a stellar nature and is unlikely to be of population I (refs 5, 6).

A further striking characteristic is that, given the velocity and galactic longitude, this object seems to be moving counter

to the general galactic rotation. This property is shared with further sources, at more modest velocities, found during the above survey.

It is a tantalising possibility that many more such sources exist near the galactic centre. This may be studied with a deep, high velocity survey. If a sufficiently large number of similar sources are found, one can then derive their space and velocity distributions and, as a consequence, calculate the gravitational potential and mass distribution close to the galactic centre. Optical studies by Minkowski⁷ on the radial velocity distribution of planetary nebulae near the centre were hampered by the interstellar extinction in that direction. Such a deep high velocity survey has the advantage of studying a well-relaxed subsystem of the central galactic bulge (no worry about non-circular motions⁸) by radioastronomical observations (no interstellar extinction⁸). Further observations are in progress.

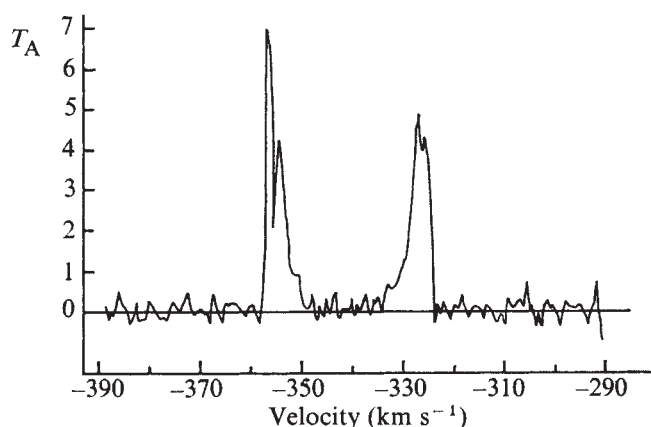


Fig. 1 1,612-MHz OH line profile of OH 0.3-0.2, observed with the 100-m telescope at Effelsberg, expressed in antenna temperature (1 K corresponds to 0.7 Jy). The frequency resolution is 6.5 kHz. Radial velocity with respect to the local standard of rest.

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Late type giants in Large Magellanic Cloud

WITH objective prisms used on a Schmidt telescope, faint late type stars may be identified with a dispersion of $1,700 \text{ Å mm}^{-1}$, at the atmospheric A band, on photographic plates sensitive in the near infrared spectral region. The required spectral classification techniques were developed by J. J. Nassau and various collaborators¹⁻³. These techniques were used by Westerlund^{4,5} with the Mt Stromolo-Uppsala 50/66-cm Schmidt telescope to survey the M-type supergiants in the Large Magellanic Cloud (LMC). Westerlund found relatively large numbers of early type M stars brighter than $m_i=13.0$ in the photometric system of Kron, Gascoigne and White⁶.

With a new objective prism⁷ which yields $6,700 \text{ Å mm}^{-1}$ at the A band attached to the 61/91-cm Curtis Schmidt telescope, late type M stars may be classified to approximately $m_i=16.5$. This prism makes possible the detection and temperature classification of M type stars later than M1, but late M stars are easier to recognise than the earlier types. The absolute visual magnitude of luminosity class III late M giants in the Solar neighbourhood is approximately -0.9 and the (M_V-M_i) colour of an M6 giant is about 3.5. Thus in the LMC whose apparent distance modulus is ~ 18.6 , it is possible to segregate and to classify these stars

since they should show up at about $m_i=14$ if they are physically similar to their galactic counterparts. Since this magnitude is appreciably higher than the limiting magnitude ($m_i=16.5$), it seemed worthwhile to carry out a search for such stars in the LMC. We present here preliminary results of such a search.

Two Kodak I N plates, hypersensitised in ammonia solutions, were used for the spectroscopic survey in the LMC. For photometric estimates two direct non-sensitised plates were used. To minimise the effects of possible variability among late M-type stars, all the observational material was gathered within an observing period of one week during October 1974. Interstellar reddening at the observed wavelengths is small, and in this preliminary survey has been neglected.

Stars of class M 6.5 and later seem to form a natural group according to the definitions of Morgan⁸, even when observed with the small dispersions given by the new objective prism. The characteristic feature of this natural group is an apparent drop in the intensity of the continuum at wavelengths longer than $8,400 \text{ Å}$. This is caused by a blend^{9,10} of TiO $8,432 \text{ Å}$ and $8,570 \text{ Å}$ bands and the VO bands at $8,575 \text{ Å}$ and $8,624 \text{ Å}$. The present study was limited to stars that belong to this group. The spectral classification technique used here yields only temperature classifications. In the absence of luminosity criteria one must rely on statistical arguments^{11,12}, which show that the overwhelming majority of stars segregated in a survey such as this are giant stars. The distributions found here according to position and to apparent magnitude leave no doubt that this is the case.

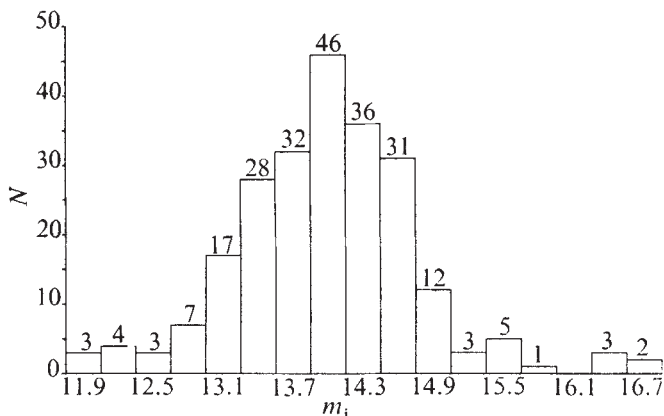
An area of 8.75 square degrees centred at RA 5 h 24 min and dec. -70.0° (1975) was surveyed. The centre of this area is close to the optical centre of the LMC and to its centre of gravity as found from the rotation of the Galaxy determined from 21-cm observations¹³.

The number of late M stars found is 803. Their surface distribution suggests a symmetrical concentration about the centre of the area where 120 late M stars per square degree are found. This surface density decreases by about a factor of 2 at a distance of 1.25° from the centre, except in the direction of the Bar, where a 20% excess of stars is found.

Apparent (m_i) magnitudes were obtained for 228 of the late M-type stars. Photometric sequences were formed by using 68 OB stars for which Lucke¹⁴ had published B and V magnitudes. The m_i magnitudes were derived from a $(B-V)$, $(V-I)$ relationship established by Blanco¹⁵. The m_i distribution shows a maximum of $m_i=14.0 \pm 0.01$. The standard deviation of the m_i distribution is ± 0.6 . This result suggests that the stars found in this survey may be similar to stars of class M6 III and M7 III in the solar neighbourhood. This coincidence is interesting in view of possible differences in the chemical abundances of stars in the Galaxy and in the LMC. It should be pointed out, that evolutionary models relating spectral classes and luminosities for late type M stars of luminosity class III are not available. Furthermore, the spectroscopic criteria employed in a survey such as the one described here may very likely contain a selection effect in which stars with strong TiO and VO features are favoured. All that one may conclude from the results presented here is that both the Galaxy and the LMC seem to show a stellar population component of late type giant stars of luminosity class III, and that these stars may be physically similar in the two galaxies. These results suggest that the LMC may have M giant stars contributing to the disk population.

This is a very preliminary report of our survey. Since it is possible that in the use of a technique as new as the one used here unexpected errors may exist, a definitive study will be made in which a large telescope aperture and a grating-prism combination as described by Bowen and Vaughan¹⁶, will be used.

Fig. 1 The 233 stars of types M6 and later plotted according to their observed apparent infrared magnitude, m_i . Magnitudes were interpolated from photoelectric measures on the BV system¹⁴ and converted to the infrared scale¹⁵. The limiting magnitude for the survey is $\sim m_i=16.5$. The stars are grouped in magnitude intervals of $0.3 m_i$; the range of magnitudes measured extends from $m_i=11.75$ to $m_i=16.85$. Maximum frequency occurs at $m_i=14.0$, which being well above the plate limit, seems to be a real effect. A group of 225 stars of types M6 and later in the interval $m_i=12.5$ to $m_i=15.8$ were used to compute the dispersion about the observed mean apparent magnitude at $m_i=14.0$; the value of this dispersion is ± 0.6 mag.



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Transient X-ray sources

In the eccentric-orbit binary model^{1,2}, a compact object is in an eccentric orbit around a more massive, slightly evolved star. If the compact object accretes mass from the stellar wind of the normal star, the accretion rate is time dependent, so that the resulting X-ray emission would show a maximum at periastron (in the absence of absorption extinction) and a minimum at apastron. It is also possible that accretion might arise from direct mass transfer resulting from contraction of the Roche lobe with decreasing primary to secondary distance. We show here that the stellar wind model, is adequate to describe the majority of transient X-ray sources.

Eccentric-orbit binaries may be produced when there is a supernova explosion in a close binary system. Usually such an explosion disrupts the binary system, but Gott³ has suggested that in a significant fraction of such supernovae the collapsed residue of the explosion (a white dwarf, neutron star, or black hole) may be retained in orbit around the runaway second star, the resultant system being a binary with high eccentricity and large space velocity reflecting its former large orbital velocity.

X-ray transients are characterised by a rapid rise, over a few days, to maximum emission, which may last for up to 4 months and then smoothly and progressively decreases. The decrease is approximately that expected by reduced accretion with diminishing stellar wind intensity as the primary-compact object distance increases from periastron. We suggest the following possible explanation for the rapid onset. The stellar wind from the primary is slowed by interaction with the magnetosphere of the compact object, and can only enter this through the 'cusps' above each magnetic pole, or through the magnetospheric tail. The X-ray emission, which then originates in the hot turbulent gas columns above the magnetic poles, depends on both the temperature and the square of the gas density. It could be that prolonged exposure to the stellar wind is required as the compact object approaches periastron before these factors reach the critical values for appreciable X-ray emission, resulting in an asymmetric light curve. Meanwhile the atmosphere of the primary will show tidal distortion, and this is likely to increase the time after periastron in which accretion can take place, so that the asymmetry of the light curve would be further enhanced.

The asymmetry was ascribed by Pacini and Shapiro² to varying absorption before and after periastron. The spectral observations of the transient A1118-61 by Ives *et al.*⁴, however, show that there is no significant change in column density, so that such an explanation is untenable, as are earlier models for transients which require large changes in the amount of circumstellar material to produce the observed light curves.

Two transients have shown a periodic behaviour with periods of the order of minutes; (A1118-61, 6.75 min; A0535+26, 104 s). Pacini and Shapiro² ascribed the periodicity to the intrinsic rotation of the compact star, which, because of the hard X-ray spectrum, is probably a neutron star (although the period of the order of minutes is rather more typical of a white dwarf). Additionally Brecher⁵ suggests that the modulation may be related to precession of the neutron star dipole magnetic field axis.

Most of the transient X-ray sources are observed to have 'hard' spectra (characterised by power law indices of ~ 0.8) that are broadly similar to other galactic X-ray sources, in particular the well understood X-ray circular orbit binaries. The eccentric binary model produces X rays by accretion in exactly the same way, thus it is expected that these two classes of objects would have similar spectra. We note, however, that the 'soft' spectrum⁶, and the identified optical counterpart of A0620-00 (ref. 7) mark it out as of a distinctive character.

Clark and Caswell⁸ have found the characteristic interval between supernovae in the Galaxy to be ~ 150 yr, comparable with recent estimates of pulsar birthrates. Thus it would seem that most supernovae produce pulsars, and an upper limit to the numbers of supernovae in binary systems which give bound runaway systems would seem to be $\sim 10\%$. Thus we would expect 10^3 eccentric-orbit binaries in our Galaxy which are runaways from supernovae which have occurred in the last 10^6 years (the time for orbit circularisation; Lea and Margon⁹). The Ariel V satellite has discovered a large number of strong transient X-ray sources since launch in October 1974. This suggests a lower limit of 6 transients brighter than the Crab X-ray source per year, and ~ 100 transients per year in our Galaxy (see ref. 10). We thus deduce that X-ray transients spend $\sim 10\%$ of their time actually on. As transients are observed to last for up to ~ 4 months, this implies orbital periods of a few years for the very eccentric orbit transients. Thus we would expect transients to reappear on time scales of up to a few years.

Two earlier models for X-ray transients may be examined in the light of the recent observations.

Many authors have compared X-ray transients with optical novae because of their similar light curves, but there is so far no convincing evidence for the occurrence of a nova close to an X-ray transient.

Fabian *et al.*¹¹ have proposed the Mira-type variable RS Cen (lying 4' outside the Ariel V error box) to be the optical counterpart of A1118-61. Their model predicts regular X-ray outbursts with a period of 164.5 d, the pulsation period of RS Cen, but Copernicus observations of this region exactly two periods before and one period after the peak of A1118-61, showed no evidence for the transient (Davison and Sanford, personal communication). The only remaining possibility is for the orbital period to be exactly three times the pulsation period of RS Cen. Although this is unlikely, it is possible to envisage systems where an expansion of the primary increases the stellar wind and so the accretion rate, giving a bright source. The contracting of the primary would then lead to the decline. This model makes several predictions: first the light curve will be determined by the rate of expansion and contraction, second if the range of the binary period is hours to weeks some indication of this may be expected in the light curve from eclipses, or, if the compact object is rotating this period would show the Doppler effect, and thirdly that the primary would be expected to show some optical brightening. Most of the well observed transients have been found to fit the synthetic light curve of Ammel *et al.*¹², and it is hard to imagine how stars with very different masses can expand and contract in identical manners. In addition no

evidence has yet been found to support the second prediction. The suggested optical identification for A0620-00 provides the only possible evidence for the third prediction, but as already noted, this seems to be a distinctive type of object.

By generalising these arguments to include Roche-lobe overflows, we envisage a possible evolutionary model for all the compact galactic X-ray sources (the only obvious exception being pulsars emitting by the synchrotron process).

A supernova explosion in a close binary system leaves a collapsed object, which may or may not remain bound to the original secondary. If mass transfer has already taken place, then the supernova occurs in the less massive star, and isotropic mass loss from a symmetric explosion cannot by itself produce an unbound runaway (see, for example, Paczynski¹³). The system could be disrupted by an asymmetric explosion, however, with the production of a runaway collapsed object which, if a neutron star, might be observable as a radio pulsar. Additionally, momentum imparted to the unexploded star by the supernova shell might contribute to the disruption of the system. Tademaru and Harrison¹⁴ have proposed that pulsars may be accelerated by the emission of asymmetric low frequency electromagnetic radiation to attain the characteristic observed velocities, which exceed 100 km s^{-1} .

Binaries that remain bound after the supernova will have an increased period and possibly a high eccentricity related to a high runaway velocity^{13,15}. The eccentric-orbit binary model for X-ray transients requires sufficient accretion near periastron (either from the stellar wind or Roche-lobe overflow of the primary) to account for the observed intense X-ray emission. Then after $\sim 10^6$ yr the orbit will have become circular and synchronised by tidal forces, so that a close (possibly eclipsing) circular orbit X-ray binary may be observed, until the Roche-lobe overflow of the primary extinguishes X-ray emission.

A further supernova in the uncollapsed companion would give, if the system remains bound, a binary with two collapsed components which may be detectable as a binary pulsar, but of the ~ 120 observed radio pulsars only one is seen in a binary system (with two collapsed components¹⁶). This implies that $\sim 1\%$ of binaries remain bound after two supernovae.

We would suggest that the source Cir X-1 is an intermediate period transient. The variability of the source and the difficulty of ascribing an orbital period have been adduced to a highly eccentric orbit with large period¹. Davison and Tuohy¹⁷ have combined their Copernicus satellite observations of Cir X-1 with earlier observations (W. A. Baity, M. P. Ulmer and L. E. Peterson, unpublished), and suggest an interval of ~ 220 d between times of peak X-ray emission. The source Aql X-1 has recently shown another 'transient-like' brightening, consistent with it also having an eccentric orbit.

The main observational consequence of the eccentric-orbit model is that all the X-ray transients would be expected to reoccur, the repetition period simply being the binary period of the system, which could be up to several years.

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Enhanced abundances of Ca, Ti and Cr in low energy cosmic rays in Skylab experiment

WE report here the results of the measurements of the abundances of Ca, Ti and Cr nuclei relative to Fe, in low energy cosmic rays in 25-100 MeV/a.m.u. interval, which were measured in a dielectric detector assembly exposed on the outside of Skylab III for 73 d. The collecting power (defined as the product of area and exposure time) of the lexan detector in this experiment was the largest so far used in free space— $1.1 \times 10^9 \text{ cm}^2 \text{ s}$ and the period of exposure from November 22, 1973 to February 3, 1974 was exceptionally 'quiet' (free from solar activity). These factors enabled us to make a reliable study of the abundance of these low energy cosmic-ray nuclei. The results on the measurements of the low energy oxygen group of nuclei in the same detector have been reported earlier¹. We found an interesting feature in the enhanced abundances of low energy Ca-Cr nuclei relative to Fe, which are different from the composition of high energy ($> 1 \text{ GeV/a.m.u.}$) cosmic rays, but are similar to those of low energy cosmic-ray nuclei observed in interplanetary space^{2,3}. In the present work, however, observations have been made down to an energy of 25 MeV/a.m.u., which is a factor of two lower than those in earlier studies.

The detector consisted of a stack of 32 lexan polycarbonate sheets, each of area 180 cm^2 and thickness 0.025 cm , covered with aluminium foil of a minimum thickness of 27 mg cm^{-2} . This module was exposed on the exterior of Skylab III at an altitude of 435 km in a circular orbit of inclination 50° for 73 d.

Data on heavy nuclei of $Z > 16$ recorded in a part of the detector were analysed. Heavy nuclei of cosmic rays on passing through the detector cause damage to its material, and give tracks visible after chemical etching when viewed through a microscope. The etched cone lengths in consecutive sheets and the positions where the particle has stopped, give information on the charge and energy of the particles. To increase the sensitivity of the lexan sheets, they were irradiated with strong ultraviolet radiation for 12 d (6 d on each side) before etching with 6.25 N KOH solution at $40 \pm 0.02^\circ \text{C}$ for 12 h. About 25 cm^2 of each of the top six sheets of lexan were scanned for tracks of nuclei of $Z > 16$.

The determination of charge was based on the well known cone length (L) against residual range (R) method⁴. The L - R plot for the tracks of $Z > 16$ are shown in Fig. 1, together with some data for $Z = 6-10$ nuclei obtained from scanning a small area of the detector. Our charge calibration is based on the assumption that: (1) the highest charge nuclei of greatest abundance are of iron, and (2) those which are most abundant for $R < 100 \mu\text{m}$ are oxygen nuclei. The calibration lines for other charges derived from the L - R values for O and Fe nuclei are shown by dotted lines in Fig. 1. The calculated and observed positions of different charges, as well as the internal consistency of the data, give support to the reliability of the charge assignments. A sample of tracks coming from the bottom side was also analysed and these data also agreed well with our charge assignments. The charge resolution for $Z \geq 20$ seen from the Fig. 1 is about $\Delta Z = \pm 1$; it is even better if one takes into account the multiple data for the same track which show good agreement. We have assigned the charge of a track to the nearest even charge, which we believe to be reliable.

The relative abundances derived from the differential fluences $d\phi/dE$ for time integrated flux are shown in Fig. 2. For a comparison, the relative abundances of galactic cosmic rays of energy $> 0.85 \text{ GeV/a.m.u.}$ are also shown, normalised to Fe. The most notable observation is the large abundance of Ca, Ti and Cr at low energies relative to Fe compared with high energy cosmic rays.

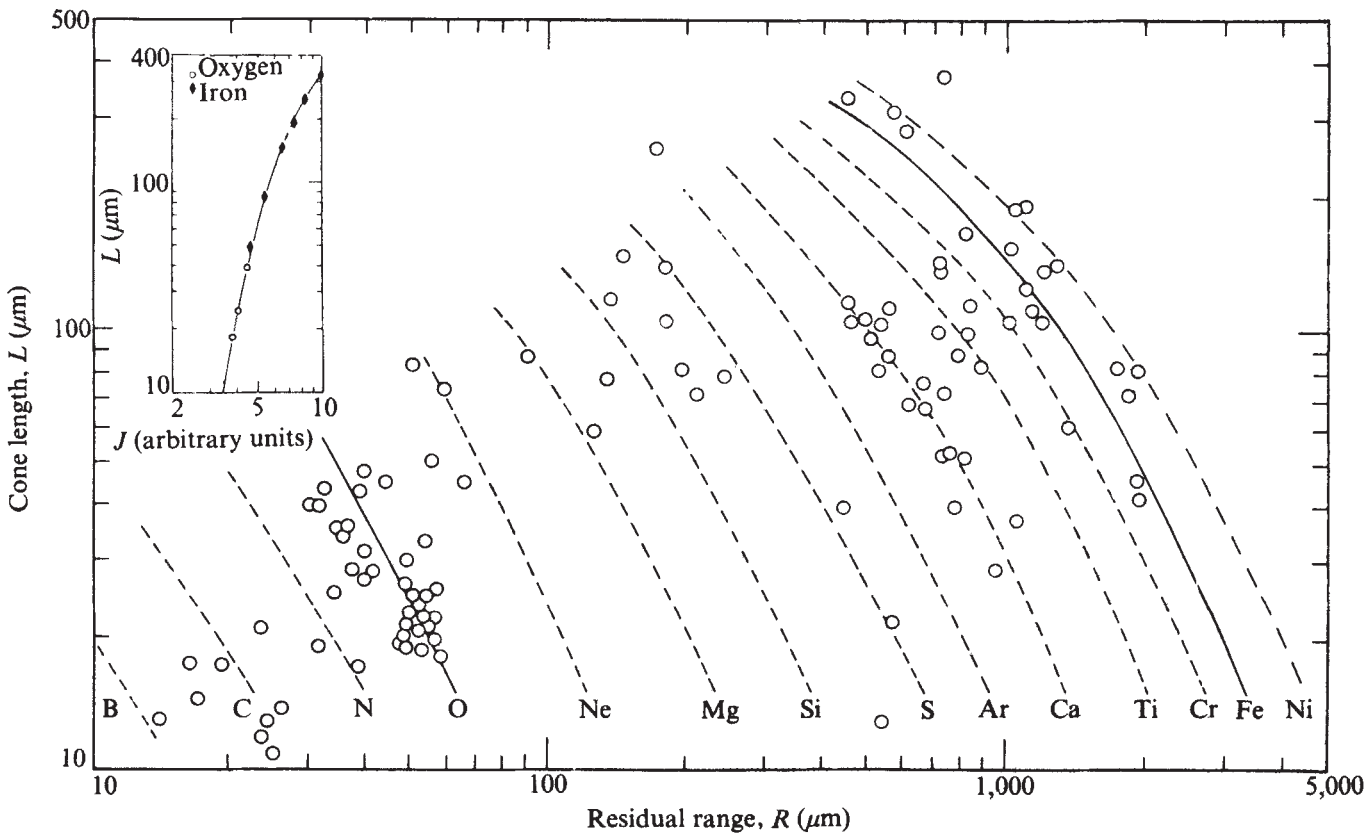


Fig. 1 L - R plot for a sample of tracks of C-Ni nuclei in the Skylab detector. The data for C-Ne are from a smaller area scan. The calibrations were carried out by fixing the O and Fe ions (solid lines; see text). The calibration curves for other charges (dotted lines) were derived from the L - J (specific ionisation) plot of O and Fe (see inset). The values of J were derived using the relation (1) given in ref. 4 with a value of $K = 10$.

Table 1 shows the abundances of Ca-Ni nuclei derived in this work in the energy interval of 25-100 MeV/a.m.u. along with the data measured by other workers^{2,3}, at slightly higher energies, on the lunar surface. The abundances in high energy galactic cosmic rays at $E > 850$ MeV/a.m.u. are also shown for comparison⁶. Individual abundances of Ca, Ti and Cr nuclei show high values (relative to Fe) as compared with high energy galactic nuclei. The striking point in our work is the value of 1.5 ± 0.5 for $(\text{Ca} + \text{Ti} + \text{Cr})/(\text{Mn} + \text{Fe} + \text{Ni})$ which is a factor of 6.5 ± 2.2 higher than the value of 0.23 ± 0.02 obtained for the high energy galactic cosmic rays. High relative abundances of Ca, Ti and Cr nuclei at low energies are also

seen to a lesser extent in the other two interplanetary experiments.

The other significant observation concerns the flux and energy spectrum of these nuclei. In Fig. 3a, we have shown the fluences of Fe-group nuclei (Cr-Ni) measured in the Skylab detectors which indicate that the energy spectrum is nearly flat in 25-100 MeV/a.m.u. interval. The interplanetary cosmic-ray flux of Fe-group nuclei has been measured^{2,3,6,7}, and the differential flux is nearly flat in the region of 20-120 MeV/a.m.u. with an average value of $(4.5 \pm 2.0) \times 10^{-8}$ particles/(cm² sr s MeV/a.m.u.) as shown in Fig. 3b. We have calculated the expected fluence at the Skylab orbit using a 5% exposure

Table 1 Relative abundances of Ca-Ni nuclei

Energy	Low energy particles			High energy cosmic rays Webber <i>et al.</i> ⁵
	Present work	O'Sullivan <i>et al.</i> ²	Fleischer <i>et al.</i> ³	
25-100		60-130	50-170	> 850 MeV/a.m.u.
Ca				
Mn+Fe+Ni	0.67±0.32	0.28±0.14	0.50±0.30	0.12±0.01
Ti				
Mn+Fe+Ni	0.29±0.19	0.09±0.06	0.56±0.35	0.06±0.01
Cr				
Mn+Fe+Ni	0.55±0.27	0.28±0.14	0.60±0.35	0.05±0.008
Ca+Ti+Cr				
Mn+Fe+Ni	1.51±0.50	0.65±0.24	1.63±0.75	0.23±0.02

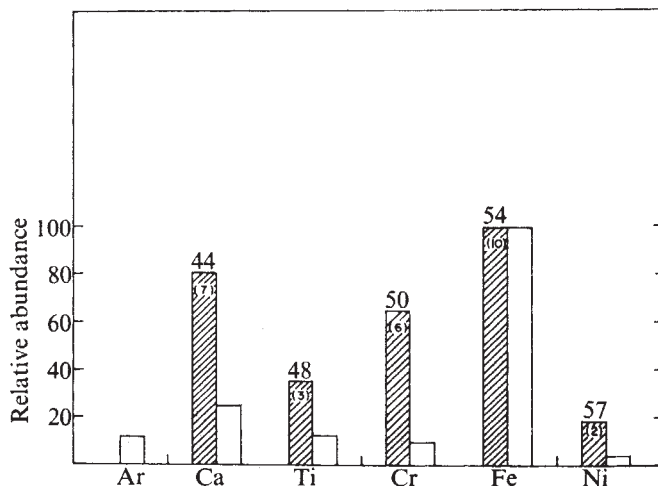


Fig. 2 Relative abundances of Ca-Ni nuclei observed at low energies in the Skylab detector compared with those of high energy cosmic rays. Note the large abundance of Ca, Ti and Cr at low energies. The average energy (in MeV/a.m.u.) and the number of events (in parentheses) are indicated. Shaded columns are our results. Open columns are galactic measurements of Webber *et al.* (> 850 MeV/a.m.u.).

factor for the average interplanetary flux. This is shown by the dotted line in Fig. 3a. If we calculate the effective exposure factor for the Skylab detector for the interplanetary flux using the vertical cutoff rigidities derived from the internal geomagnetic field of the Earth^{1,8}, we obtain the value of $\approx 1\%$. The effects of currents in the magnetosphere and the magnetospheric tail were not, however, considered in these calculations. The inclusion of these effects causes a significant lowering in the cutoff rigidity values at locations for which

Fig. 3 a, Differential fluences of Fe-group (Cr-Ni) nuclei measured in the Skylab detector. The estimated fluence at Skylab orbit, based on the average interplanetary flux of these nuclei and an exposure factor of 5% is shown by the dotted line in (a). b, The observed fluxes of these nuclei in interplanetary space, ∇ represents results of O'Sullivan, *et al.*²; $\blacklozenge$ Fleischer, *et al.*³; $\triangle$ Chan, *et al.*⁶; $\circ$ Campero, *et al.*⁷.

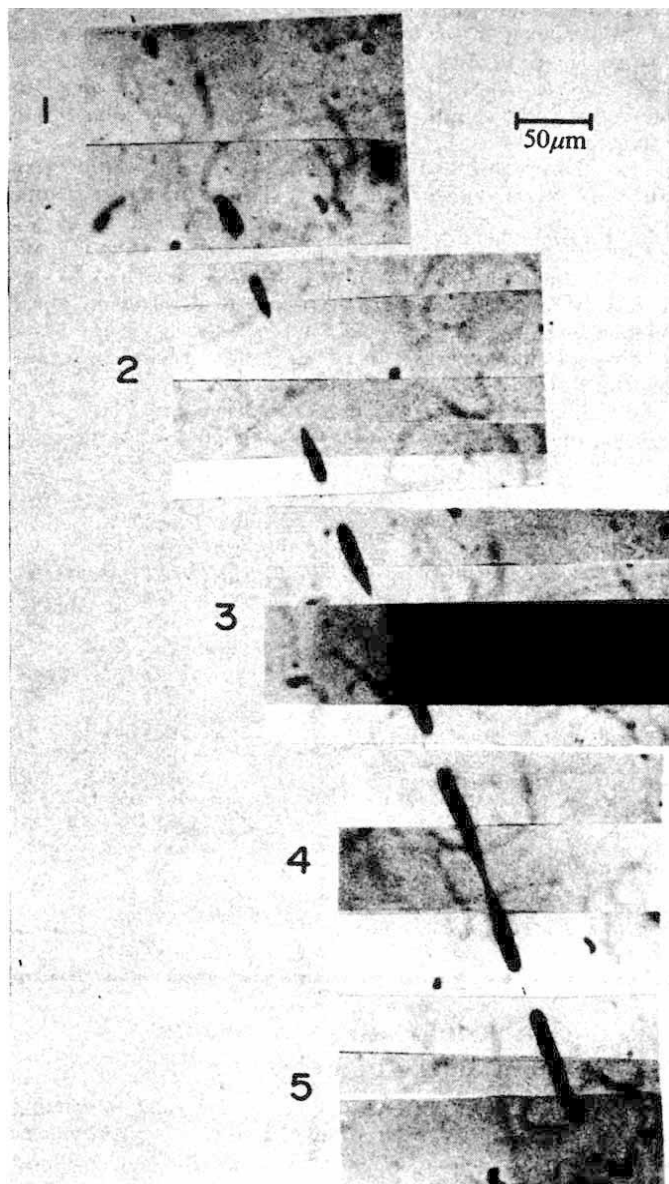
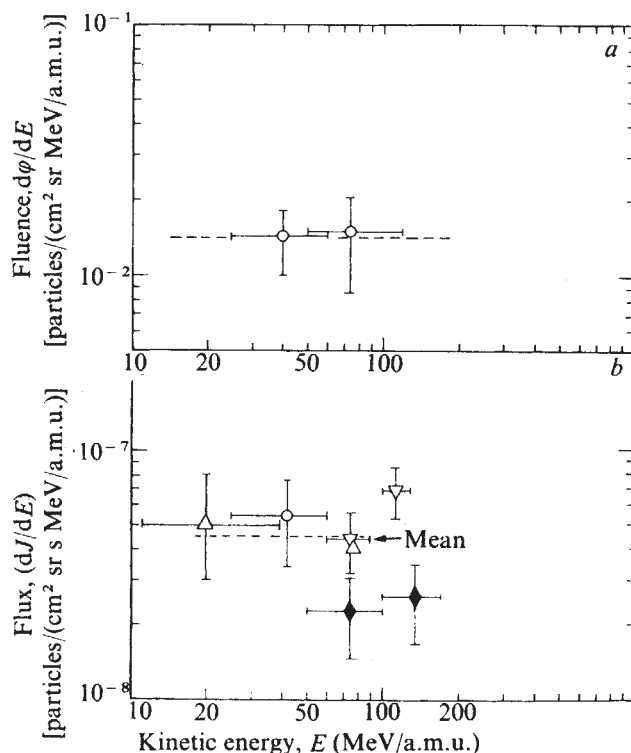


Fig. 4 Mosaic of the photomicrographs of etched tracks produced by an Fe nucleus of energy ~ 60 MeV/a.m.u. in its passage through the top six sheets of lexan detector exposed in Skylab at 435 km altitude for 73 d. The tracks are made visible by etching the sheets after irradiating them with an intense beam of ultraviolet light. Each sheet shows two tracks etched from opposite sides. Note the cones are very small in the top sheet (1). The cones in the topmost sheet (0) are still smaller and not shown in the photograph. The etch cones progressively increase in length as the particle slows down. In sheet (4) the two cones from either side merge together forming a joint double cone. The particle has come to rest in sheet (5). In the $L-R$ diagram (Fig. 1) this particle will have as many as six data points making it possible to determine the charge accurately.

internal field cutoff rigidities are ≤ 0.5 GV, as in the high latitude ($\pm 50^\circ$) passes of Skylab⁸. Observed fluences shown in Fig. 3a can therefore be understood in terms of interplanetary cosmic-ray flux.

Low energy heavy nuclei in the inner radiation belt may originate from solar wind ions of energy ~ 1 KeV/a.m.u. accelerated to a few MeV/a.m.u. in the magnetosphere by the breakdown of the third adiabatic invariant⁹. In this model, the flux of heavy nuclei above ~ 10 MeV/a.m.u. is expected to fall off rapidly. The observed spectrum of Fe-group nuclei in Skylab is almost flat in the energy interval of 25–100 MeV/a.m.u. (Fig. 3a). It is therefore unlikely that the trapped inner belt particles contribute significantly to the observed flux.

This conclusion is supported by the observed abundances of the heavy nuclei. In the solar corona the relative abundances of $(\text{Ca} + \text{Ti} + \text{Cr})/(\text{Mn} + \text{Fe} + \text{Ni})$ is ~ 0.08 (ref. 10), and the solar wind is expected to have similar composition. Our observed abundance ratio of 1.5 ± 0.5 excludes the solar origin of these particles.

It may be noted that the abundance ratio of $(\text{Ca} + \text{Ti} + \text{Cr})/(\text{Mn} + \text{Fe} + \text{Ni})$ we have observed, is similar to that measured in the low energy cosmic rays in interplanetary space as shown in Table 1, and the spectral shapes are similar (Fig. 3). We therefore conclude that the observed heavy nuclei in Skylab are mainly interplanetary cosmic-ray particles. The enhanced abundances of these heavy nuclei at low energies as compared to those at high energy ($> 1 \text{ GeV/a.m.u.}$) seems to be an important property of cosmic ray nuclei.

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The nuclear composition of cosmic rays at their source in a closed galaxy

A MAIN objective of current theories of the galactic origin of cosmic radiation has been to explain the observed nuclear composition in terms of a source composition and spallation processes occurring during the propagation from the sources to the Solar System. The so-called leaky-box model can explain the observed composition only if the cosmic rays are assumed to escape from the galaxy after having traversed a few g cm^{-2} of interstellar gas, but has trouble explaining the flux of primaries of large airshowers. These very energetic nuclei are then assumed to be of extragalactic origin, as our own Galaxy cannot contain them long enough to build up the flux to the observed value and to assure even a moderate degree of isotropy.

We construct here an alternative model to explain the observed nuclear composition, which starts with the assumption that all cosmic ray particles are of galactic origin and, once created, remain confined to the galaxy by a suitable large scale magnetic field, even at the highest energies. This implies that the particles are confined also at lower energies, so that all complex nuclei are destroyed by spallation. The particles end up as protons and finally lose their energy due to pion production, a process which requires the traversal of more than 100 g cm^{-2} of interstellar material. We next require that there is one (or more) local sources in the solar neighbourhood (within a distance comparable to the thickness of the galactic disk), contributing nuclei which on their passage to the solar system have traversed only small amounts of matter, which in a first approximation may be neglected. The assumption of a closeby source was first introduced by Lingenfelter¹.

We will further assume that the local source has the same composition as the average of the distant sources. Thus the model contains only one free parameter—the relative strength

Table 1 Source abundance of the dominant elements according to two different models (normalised to $C = 100$)

	Equilibrium plus local source	Leaky box
H	~ 0	50,000
He	2,750	2,600
C	100	100
O	100	107
Ne	17	16
Mg	22	23
Si	16	20.4
Fe	13	20.5

of the contribution of the local source compared with that of the background flux.

We can now calculate the composition of the source from the observed fluxes of the elements. The calculation is based on partial spallation cross sections taken from a compilation by N. J. Westergaard (unpublished) which includes recent results obtained at the Bevalac accelerator

$$S_n = \frac{\sigma_n F_n - \sum_{j>n} \omega_{nj} (F_j - K S_j)}{1 + \sigma_n K} \quad (1)$$

where S_n is the relative source strength for the acceleration of the species n , F_n the observed flux, σ_n the total destruction cross section, ω_{nj} the partial production cross section of species n from species j . K is a parameter describing the overall strength of the local source.

This formula has to be modified for $n \leq 4$, since α -particles as well as protons (or neutrons which decay into protons) occur both as residue in the complete destruction of a complex nucleus and as evaporation particles accompanying its partial destruction.

The corresponding (secondary) flux of nucleons in the combined hydrogen plus helium component from these two contributions is given by

$$\sum_{i>4}^1 (F_i - K S_i) \left[A_i \sigma_i - \sum_{n>4}^{i-1} A_n \omega_{ni} \right] \quad (2)$$

where A_n is the mass of species n and the attenuation cross section for protons is given by²

$$\Sigma = 35 (1 - \langle \eta \gamma \rangle) \text{ mbar}$$

We use $\eta = 0.65$ as the fraction of total energy retained, (that is, not lost to pions) in p-p collisions between 3 and $\sim 7 \text{ GeV}$ kinetic energy and $\gamma = 0.66$ as the integral exponent of the published proton spectrum in the same energy interval. Thus

$$\Sigma \sim 8.7 \text{ mbar}$$

(Between 10 GeV and 30 GeV the incident proton spectrum is steeper and Σ increases to $\sim 14 \text{ mbar}$.) Since the lifetime of protons is much longer than that of helium nuclei ($\Sigma \ll \sigma_{\text{He}} = 104 \text{ mbar}$), the lifetime of the combined proton and α -component is essentially determined by the cross section Σ .

Table 1 gives the relative abundance of the most prominent elements in the cosmic ray sources as derived from the two different models. The calculation is based on the observed elemental flux in the Solar System at approximately 3 GeV/n . The parameter K , which characterises the relative contribution from the local source is obtained by minimising

$$\sum_{m=\text{Li}}^{11} S_m$$

that is, the amount of Li, Be, and B in the source. This leads to $K = 0.02 \text{ mbar}^{-1}$. The first column represents the calculated source composition as derived from the present model. The second column represents the composition as derived by

Shapiro *et al.*³. Their calculation is based on the leaky-box model in which the cosmic rays are allowed to escape from the galactic disk after having traversed a few g cm^{-2} of interstellar gas. The path length distribution is assumed to be a truncated exponential. The 'equilibrium plus local source' model differs from the leaky-box model mainly in that protons are not emitted by the cosmic ray sources, but are disintegration products of the heavier nuclei.

The local source accounts for a fraction f of the observed flux, given by

$$f = \frac{K\sigma_n}{1 + K\sigma_n};$$

it differs for each primordial element:

$$f = \begin{cases} 93.4\% & \text{for Fe} \\ 88.6\% & \text{for Mg} \\ 85.3\% & \text{for O} \\ 67.5\% & \text{for He} \\ 0\% & \text{for H} \end{cases}$$

This means that it is responsible for about 15% of the incident nucleons of a given velocity around 3 GeV/n. Thus, the present model predicts some anisotropy for the heavy nuclei, but probably not enough to disturb significantly the overall isotropy of cosmic radiation in the Solar System.

The 'equilibrium plus local source' model can account for the observed fluxes of all those elements which are assumed to be present in the source as well as for the observed flux of H, Li and Be. It predicts somewhat too little B and elements in the sub-iron group, that is with atomic numbers between 19 and 25.

A first step in refining the model is to assume that the particles from the local source have traversed on the average not zero but a small amount of matter before reaching the Solar System. This introduces a second free parameter into the model. The importance of the local contribution suggests that this matter is located in the source itself, or in its neighbourhood, and that, therefore, the path lengths are fairly narrowly distributed around a mean value x which probably decreases with increasing particle rigidity. The correct ratio between the observed flux of iron and elements of the sub-iron group at 3 GeV/n requires $x \sim 1\text{--}2 \text{ g cm}^{-2}$, and the expected decrease of x with increasing rigidity would account for the observed increase of this ratio with energy.

The discrepancy between the predicted and observed flux of Boron suggests that particles from the local source have perhaps undergone some processing by spallation reactions, which favour the removal of a single nucleon from a heavy nucleus, such as a strong irradiation by γ rays, or possibly some interactions in the source region during the early stage of the acceleration process.

The observed energy dependence of the ratio between light nuclei (Li, Be, B) and medium nuclei (C, N, O) seems easily reconcilable with the model, if one assumes that the spectrum of the local source is slightly flatter than that of the average of the distant sources. An important prediction, if one accept this explanation, is that the proton spectrum should be steep and follow that of light nuclei, rather than C, N, and O, because the protons are not primordial but secondary.

General arguments make it seem likely that the model can account for the observed isotopic abundances of H, He, and Li; and that ^{10}Be should be practically absent at low energies, in agreement with data recently obtained by the Chicago group and others. In contrast, some shorter-lived radioactive isotopes could be present if they come from the local source. The observed positron flux might be reconciled with the model using acceptable values for solar modulation parameters and for the gas and field density of the galactic confinement volume, which

necessarily will be much larger than the volume of the galactic disk. The expected γ -ray flux is not changed, since it depends on the flux of nucleons in the galaxy, not on their lifetime.

Predictions, based on this model at energies below the range considered here, will have to take into account the large difference in energy loss by ionisation of the old and the new particles in the observed flux.

The main advantage of the model seems to us to be that it makes a galactic origin of cosmic rays possible at all energies, and thus abolishes the need for the extragalactic cosmic ray component which was supposed to dominate in the very high energy region. It gives a reasonable account of the curious ratio of hydrogen to complex nuclei in the cosmic radiation which differs from the ratio in interstellar matter and also from the ratio in ordinary stars and on the surface and in the interior of supernovae. Also the total amount of energy necessary to maintain the cosmic ray intensity in the galaxy is appreciably reduced, since it will no longer be necessary to replenish the entire flux every ~ 3 Myr.

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Mantle-plume model for the origin of Archaean greenstone belts based on trace element distributions

TRACE element studies of Archaean greenstone volcanics indicate the existence of two major types of tholeiite¹⁻³: depleted Archaean tholeiite (DAT) showing flat rare earth element (REE) patterns similar to modern rise tholeiites and enriched Archaean tholeiite (EAT) exhibiting slight light REE enrichment and generally greater concentrations of LIL (large-ion lithophile) elements than DAT. Included in the DAT category are komatiitic tholeiites. DAT and associated ultramafic flows and sills seem to make up from 50 to 90% of Archaean greenstone successions. EAT becomes relatively more abundant at stratigraphically higher levels and in the upper parts of minor volcanic cycles within greenstone successions. Although overall EAT is generally less abundant than DAT, it accounts for up to 50% of some greenstone sections^{2,4}. DAT seems to be absent in the upper 10–20% of most greenstone successions.

In terms of trace element distributions, andesites in Archaean greenstone belts fall into three categories¹⁻³: depleted Archaean andesite (DAA) exhibits flat REE patterns, negative Eu anomalies and low LIL-element content; low-alkali Archaean andesite (LAA) shows minor light REE enrichment and low LIL-element content; and high-alkali Archaean andesite (HAA) shows light REE enrichment and high LIL-element contents. Andesites are minor in most greenstone belts (western Kenya being an exception⁵), becoming relatively abundant only in the upper 30% of greenstone successions. HAA exceeds LAA in relative importance only in the upper 10% of most sections. DAA has so far only been reported from the Abitibi belt in Quebec where it is associated with DAT also exhibiting negative Eu anomalies¹.

Siliceous volcanic rocks (dacite, rhyodacite, rhyolite) in Archaean greenstone belts fall into two groups based on trace element contents: depleted siliceous volcanics (DSV) exhibit heavy REE and Y depletion, whereas undepleted siliceous volcanics (USV) do not. Both groups exhibit en-

Table 1 Summary of trace element models for the origin of Archaean greenstone and granite magmas

Tholeiite	
DAT	25–35% melting of lherzolite or 10–20% melting of plagioclase peridotite
EAT	45–55% melting of eclogite
Andesite	
DAA	10–20% melting of plagioclase peridotite
LAA	20–30% melting of eclogite
HAA	10–20% melting of eclogite
Siliceous rocks	
DSV and tonalite	10–20% melting of eclogite
USV and granodiorite	30–60% melting of siliceous granulite
High-K granite	70–80% fractional crystallisation of granodiorite

From data in refs 1–4, 7 and 8.

richment in transition metals when compared with younger analogues. Siliceous volcanics, in general, make up only a minor amount of the greenstone successions (< 10%) and are often restricted (with the exception of dikes) to the upper 10–20% of the section. If existing analyses are representative, DSV exceeds USV in abundance. Greenstone belts are intruded by a variety of granitic plutons. Although such granitic rocks are not adequately described in most areas, they are reasonably well known in the Barberton region of South Africa^{5,6}. In this area, the oldest intrusions are tonalite diapirs followed by voluminous granodiorite batholiths and finally by minor, anorogenic high-K granites. The tonalites have trace element patterns similar to DSV and the granodiorites to USV. The high-K granites are characterised by high LIL-element content and negative Eu anomalies.

Trace element studies have recently proved valuable in testing various mechanisms of partial melting and fractional crystallisation for the origin of the magmas that produced the igneous rocks in greenstone-granite terrains^{1,2,4,7,8}. Relying chiefly on those trace elements least susceptible to mobilisation during alteration and low grade metamorphism (namely, Y, Zr, REE, Co, Ni and so on), it is possible to eliminate many models for magma origin and to focus on others. Although fractional crystallisation models are allowed or necessitated in some instances^{7,8}, crystal-liquid equilibria during partial melting seem to have left the strongest imprint on trace element distributions in greenstone volcanics and associated granitic rocks. The most acceptable trace-element models for each of the volcanic and granitic rock types found in Archaean greenstone belts are summarised in Table 1.

Any model for the origin and development of Archaean greenstone belts and associated granitic terrains must account for the following features: (1) the dominance of an ultramafic magma source area during the early and intermediate stages of greenstone belt evolution; (2) the increasing importance of an eclogite source area during the later evolutionary stages; (3) partial melting of siliceous granulite during the late stages to produce granodiorite and high-K granite magmas; (4) an overall decreasing degree of partial melting in the source regions with time, and (5) the origin of minor volcanic cycles which seem to necessitate replenishment of both ultramafic and eclogite sources.

One model that seems capable of explaining these features is a mantle-plume model⁸, the overall features of which are described here. A mantle plume with a region of extensive melting at its top ascends, penetrates the lithosphere, and spreads laterally producing an overlying rift system which is partially filled with DAT and ultramafic magmas derived from the plume. It is not critical to the model whether earlier sialic crust existed or not. A large volume of these magmas are intruded into the lithosphere above and adjacent to the plume and, because of the steep geothermal gradient, the mafic magmas crystallise into garnet granulite

mineral assemblages. As the plume begins to cool and subside, the overlying lithosphere begins to sink. A decreasing geothermal gradient results in the inversion of garnet granulite above the plume to eclogite. Continued sinking of the eclogite results in its partial melting giving rise to EAT magmas and, with a further decrease in temperature, to minor volumes of andesite and more siliceous magmas. Some of the latter rise as tonalite diapirs. Further sinking in response to plume subsidence leads to partial melting of siliceous granulites in the upper part of the lithosphere (lower crust) thus producing granodiorite magmas and, through fractional crystallisation, high-K granite magmas. The siliceous granulites may represent the lower portion of an older sialic crust upon which greenstone belts erupted and/or they may have crystallised from magmas of intermediate composition emplaced at appropriate depths in the lithosphere overlying the plume.

Thus the plume model provides the necessary changing source region composition for magma generation with time as well as a dropping geothermal gradient. Minor volcanic cycles within greenstone belts can be produced by cyclical plume activity, with each cycle decreasing in intensity with time. Renewed plume activity during a cycle replenishes the DAT source (directly from the plume) and, by intrusion and later recrystallisation of DAT magma, the eclogite source. It should be pointed out that the flat REE patterns and negative Eu anomalies in mafic and intermediate volcanics from the Abitibi belt may require a plagioclase peridotite source^{1,3}. In any case, eclogite is not an acceptable source rock for any of the volcanics analysed from this belt.

The linear arrays of greenstone belts in some areas (such as the Superior Province) and irregular distribution in others (such as Rhodesia) may have developed in response to plume patterns which in turn reflect differences in Archaean structural inhomogeneities in the upper mantle. Variations in the relative abundances of volcanic rocks in greenstone belts in different geographic locations^{3,4} can also be accounted for by a plume model. Large, relatively hot plumes and/or a thin lithosphere would favour production of greenstone successions in which DAT greatly dominates, such as the Barberton and greenstone belts in western Australia. Small, relatively cool plumes and/or thick lithosphere (especially if composed largely of eclogite) on the other hand, would favour the production of relatively greater volumes of EAT, intermediate, and siliceous magmas such as characterise the Midlands belt in Rhodesia and the greenstone belts in western Kenya.

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Phytenic acid in sediments

PHYTANIC acid and several lower isoprenoid acids are found in the marine food chain and in sediments. The mechanism of the degradation of phytol to phytanic acid is still uncertain. At least two pathways have been proposed and shown to occur in some cases¹. One by way of dihydrophytol, another by phytenic aldehyde and phytenic acid. Phytenic acid was postulated recently² as intermediate in the degradation of phytol in sediments and was mentioned among isoprenoid compounds

in Mud Lake sediment³. It is formed from phytol thermocatalytically in oxic conditions⁴.

We present and discuss the occurrence of phytanic acid in a lacustrine detritus layer and in a marine diatomaceous ooze. Since the analytical behaviour of the acid is peculiar, care must be taken that its presence is not overlooked. Therefore chemical and chromatographic data are discussed at length.

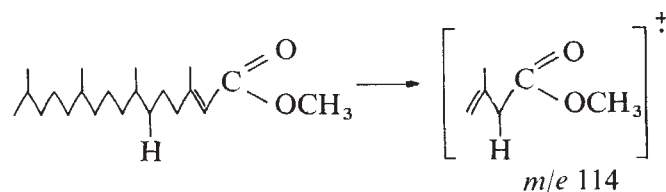
The lacustrine detritus predominantly consists of remains of *Fontinalis antipyretica* in an apparent state of degradation. It was sampled from Lonnekermeer (Twente, Holland)⁴ which is a small lake considered to be in a non-polluted state. The wet detritus was extracted with chloroform and the extract subjected to silica-gel column chromatography, using gradient elution with pentane-ethyl acetate. The acid fraction was methylated⁵ and the resulting methyl esters were separated into saturated, mono-unsaturated and poly-unsaturated fractions by means of preparative AgNO₃-thin layer chromatography (TLC) (10% AgNO₃ on silica gel), using hexane-diethyl ether (97.5:2.5 v/v) as an eluent. Methyl phytenate was shown to be present in the saturated methyl ester fraction as indicated by its gas-liquid chromatography (GLC)-retention time and mass spectrum, obtained on a Varian MAT 111 GC/MS instrument using a 30-m capillary column (0.25 mm inner diameter) coated with SP 2250. Helium was used as carrier gas.

The marine sediment is a diatomaceous ooze from the inner shelf of S.W. Africa (Walvis Bay). Four sediment sections were investigated (B41: 3–28 cm; B16: 28–53 cm; B26: 53–78 cm; B1: 78–103 cm) from a core KF6 (23°15'S and 14°05.5'E). The sediment was handled as described earlier⁶. Freeze-dried sediment was extracted with 0.1 N KOH in 95% methanol-benzene (4:1 v/v)⁷. After acidification a total lipid extract was obtained. After selective extraction of free fatty acids from the extract⁸, the neutral fraction was separated by preparative thick layer chromatography on silica gel⁹. Methyl phytenate was present in the TLC-cut containing the aromatic hydrocarbon fraction (see Table 1).

Phytanic acid was found to be present in the four sediment sections of core KF6. No phytanic acid was produced from phytol during the experiment, as was shown by applying the complete analysis to a sample of pure phytol.

Phytanic acid was synthesised from purified natural phytol (Merck), by subsequent oxidation with MnO₂ and Ag₂O¹⁰. The reaction mixture was methylated⁵ and methyl phytenate was isolated by preparative thick layer chromatography on silica gel⁹. GLC analysis indicated the presence of one methyl ester (ECL value 18.45). Infrared measurement shows a carbonyl absorption at 1,720 cm⁻¹ (CCl₄) characteristic of an α,β -unsaturated ester, and an absorption of 1,650 cm⁻¹ which points to a substituted double bond conjugated with a carbonyl function. Nuclear magnetic resonance (NMR) measurement (Varian H60) reveals a signal for the olefinic proton at δ 5.6 p.p.m. (CCl₄).

The mass spectrum shows a molecular ion peak at m/e 324 (Fig. 1c). Some characteristic fragmentation peaks are m/e 293 ($M^+ - 31$), m/e 292 ($M^+ - 32$), m/e 250 ($M^+ - 74$) and m/e 114 (probably a γ -H-McLafferty rearrangement ion).



These data agree well with the structure of (E)-3,7,11,15-tetramethyl hexadec-2-enoic acid methyl ester. The chromatographic data of this compound presented in Table 1, were obtained by silica-gel TLC⁹ and by AgNO₃-TLC on silica gel using hexane-diethyl ether (97.5:2.5 v/v).

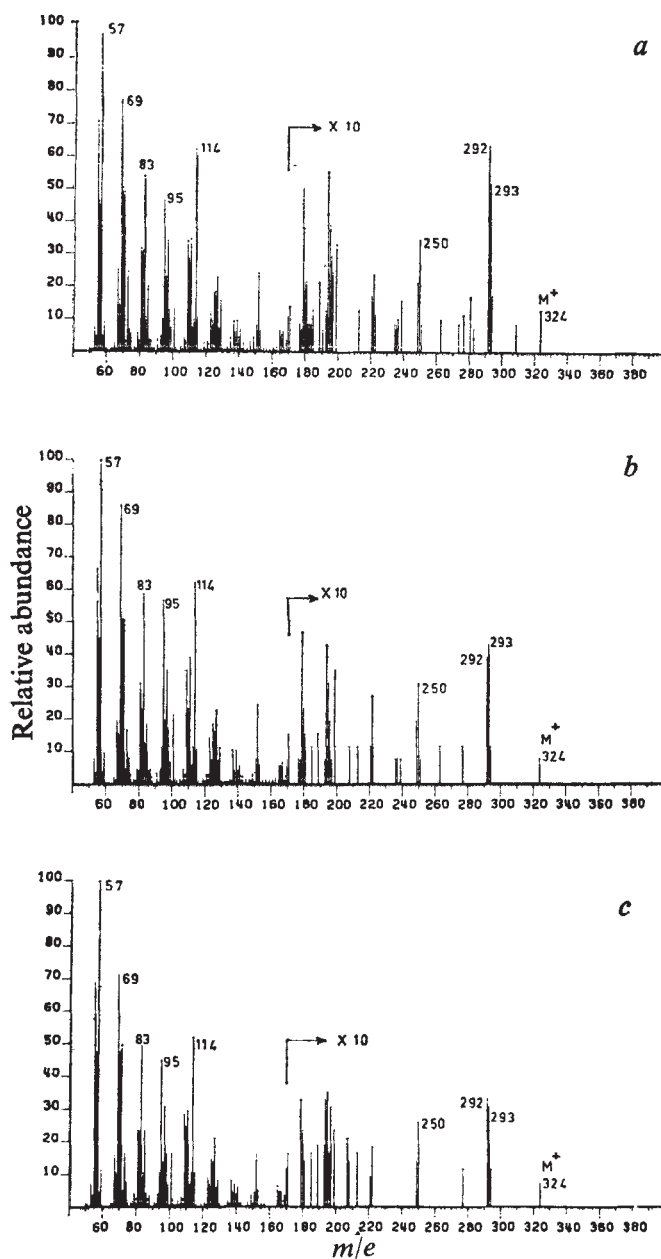


Fig. 1 Mass spectra of phytanic acid methyl ester. *a*, From lacustrine detritus; *b*, from marine diatomaceous ooze; *c*, synthetic standard compound

Figure 1 presents the mass spectrum of the methyl ester from both sediments tentatively identified as methyl phytenate, and the mass spectrum of the standard, which prove to be almost identical. Because of the peculiar chromatographic behaviour (see Table 1), it becomes clear that definite identification can only be based on the mass spectral data obtained by GC/MS.

Use of AgNO₃-TLC and GLC methods only, can easily lead to a misinterpretation of the methyl phytenate peak which is incorrectly considered a saturated ester. Another hazard is the apparent ease of esterification of phytanic acid. If alkaline alcoholysis is used to obtain the fatty acids from sedimentary, plant or animal material, the phytanic acid shows up in the neutral fraction as the ester (see Table 1).

It is known that esters can easily be formed when sediments are extracted with methanol containing solvents in the presence of clay surfaces¹². In our case the TLC and AgNO₃-TLC data, the equivalent chain length (see Baxter¹¹) and the mass spectrum both indicate that for both sediments we have (E)-3,7,11,15-tetramethyl hexadec-2-enoic acid. This compound can be seen as an intermediate in the conversion of phytol to other isoprenoid

Table 1 Chromatographic data of methyl phytenate as compared with standard compounds

Compound	R_f TLC	R_f Ag ⁺ -TLC	R_f Ag ⁺ -TLC ¹¹	ECL value SP 2250	Relative retention time SE 30 ¹¹
Methyl phytenate*	0.70	0.29	0.53	1,722	1.00
(E)- Δ^2 -methyl phytenate	0.70	0.27	0.53	1,845	1.56
(Z)- Δ^2 -methyl phytenate			0.62		1.07
Methyl stearate	0.63	0.22	0.47	1,800	1.12
Methyl oleate	0.63	0.09	0.26		
Normal alkanes (C ₁₀ -C ₃₆)	0.90				
Aromatic hydrocarbons (for example phenanthrene, pyrene)	0.7-0.8				

* Obtained from Appl. Science Lab.

compounds^{2,4,13,14}. The fate of phytol in sediments and organisms has been investigated using U-¹⁴C-phytol^{4,14-16}. It is generally agreed that phytanic acid is the key intermediate in the catabolism of phytol. This compound has been found in a variety of organisms¹. Phytanic acid is degraded further by α - and β -oxidation. The pathway of phytol to phytanic acid is less well understood. Some authors report dihydrophytol as the important intermediate, others have found phytenic acid.

Dihydrophytol is produced by certain microorganisms. Patton and Benson¹⁵ have shown its formation in the rumen of cows, a very reducing environment. It is also formed from phytol in a lacustrine sediment¹⁶, and Sever and Parker¹⁷ have shown its presence in a variety of marine sediments. Phytenic acid is found as intermediate in tissues of rats and mice¹⁴, but its production from phytol by microorganisms has not yet been shown to occur.

There seems to be some relationship between phytenic acid, dihydrophytol and phytol in sediments. In both the lacustrine detritus and the diatomaceous ooze we found no dihydrophytol, but did find phytol in the fatty alcohol fraction (unpublished). Unfortunately no fatty alcohols were determined in Mud Lake sediment³. If the occurrence of phytenic acid and dihydrophytol is mutually exclusive the presence of one of these compounds may point to certain sedimentary circumstances and/or groups of organisms acting on the organic matter. Seen in this light both compounds have considerable diagnostic value in the organic geochemistry of recent sediments.

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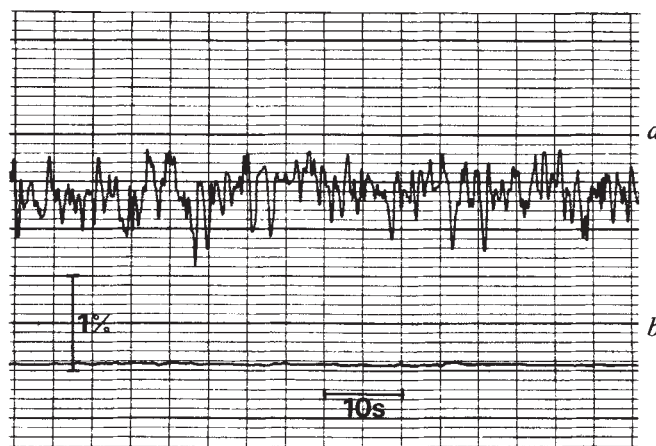
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Fluctuations in the light output from gas-filled incandescent lamps

THE gas-filled, incandescent tungsten lamps used as photometric and radiometric standards have good long term stability, but their performance is frequently marred by fluctuations in light output resulting from changes in the convective cooling of the filament. Typical fluctuations are shown in Fig. 1a, which is a record of the output from a ribbon filament lamp of the type used at the National Physical Laboratory (NPL) as a standard of spectral radiance (Fig. 2a). In this situation, an accuracy of 0.1% (a common requirement in standards work) can only be obtained by averaging for several seconds, a tedious restriction where a large number of measurements is required.

It is not possible to dispense with the gas filling without incurring excessive evaporation of tungsten from the filament, but the fluctuations might be reduced if the convective gas flow within the lamp could be stabilised aerodynamically. We therefore studied the pattern of the gas flow by the use of a Schlieren system in conjunction with a number of special lamp envelopes having optically flat windows. To facilitate quick and easy access for modification inside the lamp, the envelopes were left unsealed and made demountable, the experiments being carried out in air at atmospheric pressure. As tungsten would have oxidised in these conditions, filament models were made from platinum or palladium and operated at relatively low temperatures, below their melting point. A number of different structures were examined and it was found that the fluctuations were effectively suppressed by the provision of a cylindrical chimney some 25 mm in diameter and 60 mm long mounted

Fig. 1 a, Record of light output against time for a conventional gas-filled, incandescent, tungsten ribbon lamp. b, Record of light output against time for a modified lamp.



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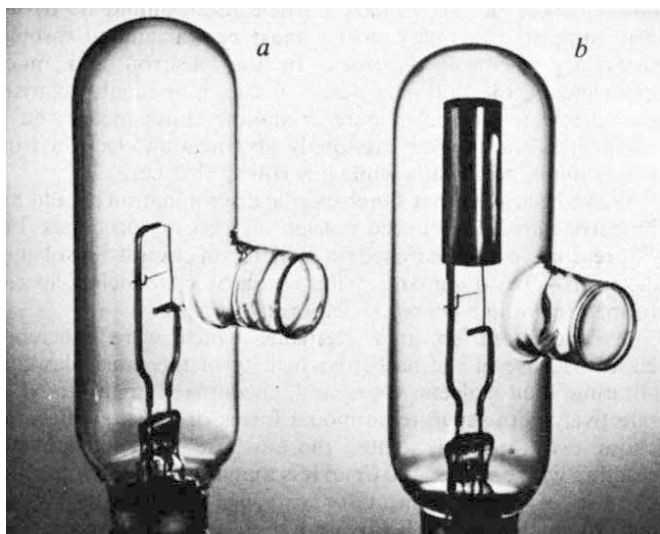


Fig. 2 *a*, Gas-filled, incandescent, tungsten ribbon filament lamp used at the NPL as a standard of spectral radiance. *b*, The lamp modified by the addition of a cylindrical chimney above the filament.

vertically just above the top of the incandescent filament. It was, of course, necessary to confirm these findings in actual lamps, and a number of argon-filled, tungsten ribbon lamps of the conventional NPL pattern, but incorporating the chimney, were made to our specification by the Hirst Research Laboratory of the General Electric Company (Fig. 2*b*). The effect of the modification on the light output is shown in Fig. 1*b*.

Our Schlieren observations showed that in the unmodified lamp, the plume of hot gas which rises from the base of the filament tends to wander across the filament in a manner reminiscent of a guttering candle flame. The chimney seems to separate the central rising gas column from the cooler gases flowing back down the outer parts of the lamp envelope, giving smooth and stable gas flow in the region of the filament. The dimensions of the cylinder did not seem to be critical for the lamp investigated and it seems likely that the fluctuations in other types of gas-filled incandescent lamps could be suppressed in a similar way.

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Preliminary palaeoenvironmental implications of pollen analysis of Middle Breccia from Sterkfontein

SUCCESSIVE excavations at the Sterkfontein site, about 50 km west of Johannesburg, have yielded numerous vertebrate remains, including those of *Australopithecus* and possibly *Homo*, together with some artefacts. The geology and stratigraphy of Sterkfontein have been described by Butzer¹ and by Partridge², and Vrba^{3,4} has discussed the chronological and palaeoecological implications of the fossil mammals found there.

Robinson⁵ divided the sequence into a Lower Breccia, later referred to as the STS unit, containing vertebrates and *Australopithecus*, a Middle Breccia containing possible *Homo* and numerous artefacts, and an Upper Breccia. T. C. Partridge

(personal communication) suggested recently that the entire sequence should be included under the term "Sterkfontein Formation", divided into a number of members and beds. The Sterkfontein Formation sediments comprise red breccias and siltstones, held together by travertine which is most probably a secondary cement.

The relative scarcity of pollen grains in these sediments made earlier attempts at extracting them difficult, whereas the high carbonate content and the red coloration apparently prevented any later attempts.

The pollen extraction described here was based on the idea that the sediments had been washed into a previously existing cave after having been oxidised outside the cave, and that the travertine represents a later stage of carbonate precipitation that could not interfere much with the pollen preservation. This was also encouraged by positive results obtained while analysing cave deposits from Israel.

A sample of ~2.5 kg of sediment was collected from the Middle Breccia (unit SE, or member 5 of the Sterkfontein Formation), and underwent a long process of preparation. Basically this process involved total dissolution of the carbonates with 10% HCl, partial dissolution of the silicates and clays with a 40% HF, and subsequent mineral gravitational separation using ZnCl₂ solution in 10% HCl, with a specific gravity of 1.9. The residue was washed with 10% KOH and water and mounted in glycerine jelly. The results were surprising, and thousands of pollen grains were recorded in the slides. For comparison, a sample of recent soil from the site was also prepared.

About 400 pollen grains have been counted in the slides prepared from the Middle Breccia sample, and were divided into two groups on the basis of their state of preservation (Fig. 1): the first assemblage, which yielded 138 grains, comprised heavily corroded and carbonised fossils. The second assemblage, which yielded 262 grains, comprised only slightly corroded and

Fig. 1 Comparison between badly and better preserved pollen from the Middle Breccia, Sterkfontein ($\times 930$). *a*, *b*, Gramineae. *c*, *d*, *Podocarpus* spp. *e*, *f*, Compositae.

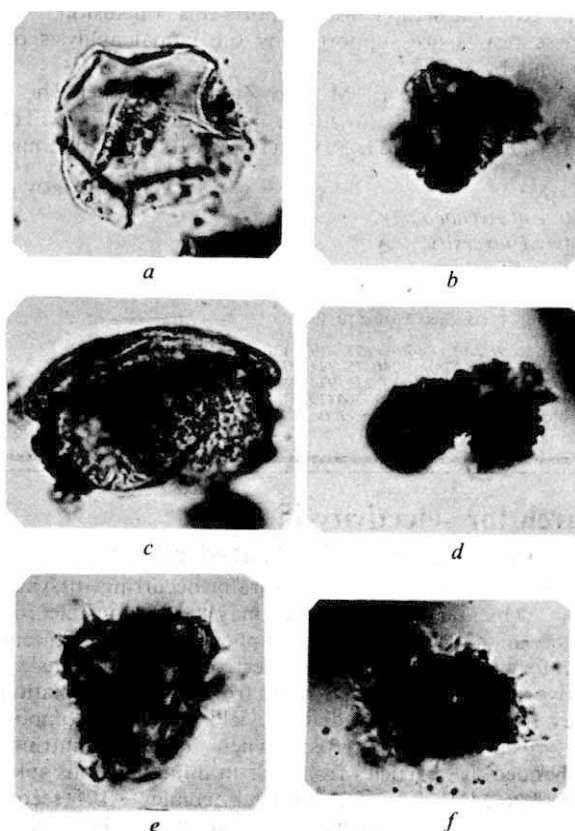


Table 1 Pollen spectra of samples from Sterkfontein (percentage of the total for each group)

	Middle Breccia		Recent
	Badly preserved	Better preserved	
Gramineae	84	48	37
Compositae	11	5	2
Fern spores	3	9	1
<i>Podocarpus</i>	1	10	1 (other than fossils)
<i>Euphorbia</i>		16	
Cyperaceae		3	12
<i>Rhus</i>		4	33
<i>Pinus</i>			2
<i>Olea</i>			5
Chenopodiaceae		1	5
Unidentified	2	3	1
Others		3	1

carbonised material. The Recent material was much better preserved. Table 1 summarises the percentages of the first and second groups, in comparison with the Recent spectrum.

A preliminary suggestion is hereby proposed as to the palaeoenvironmental implications of the two pollen spectra from the Middle Breccia: the badly preserved, apparently older material, represents the environment at the time of accumulation of the cave sediments, including the bones and artefacts, whereas the second, better preserved material was brought into the sediments by the percolating water during a later stage, a process that has been responsible for the travertine deposition and part of the secondary cementation.

Each of the two spectra is totally different from the Recent one. It is suggested, based on the predominance of Gramineae pollen and the scarcity of other species, that the Middle Breccia was formed when the surrounding area was a vast, open grassland, and probably when the climate was drier than at present. The travertine formation took place when the climate was much more humid, and probably also warmer, than at present. This is indicated by the *Podocarpus* and *Euphorbia* assemblage that appears today towards the lower, warmer and more humid areas of the Transvaal. The single grain of *Rauvolfia* that was encountered in the sample also supports this conclusion.

These results are supported by the faunal analysis of the sediments by Vrba^{3,4}.

I thank Professor E. M. van Zinderen Bakker who initiated and encouraged the study, and Professor P. V. Tobias, Drs T. C. Partridge and E. S. Vrba for discussions and comments.

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Search for selectivity in interactions of chiral solvated electrons

ULBRICHT'S suggestion¹ that naturally occurring dissymmetry at the elementary particle level may be responsible for the chirality of molecules in the biosphere has been subjected to laboratory tests using polarised electrons^{2,3} and muons⁴. Some positive results are claimed to result from β^- and β^+ radiations^{2,3}. Garay found a higher radiation yield for the decomposition of D-tyrosine than for L-tyrosine when subjected to intrinsically 'left-handed' β^- particles from ⁹⁰Sr in dilute aqueous solution. Since 2.26-MeV β^- particles each create $\sim 10^6$ secondary electrons during their energy degradation, the direct chemical

consequences of the primary particle itself should be trivial. This suggests that dissymmetry must be transmitted through secondary chemical reactions. In the positron and muon experiments the ultimate fate of the individual polarised particles were studied in pure crystalline chiral media and in these cases the particle eventually abstracts an electron from the medium, principally while it is still epithermal.

It can be argued that stereospecific discrimination should not be particularly pronounced in such high energy processes. For this reason we have searched for evidence of chirality in solvated electrons—the dominant primary reactive reducing species formed from high energy radiations.

We produced solvated electrons which were inherently chiral because of the particular helicity of the molecules constituting their solvent cage, and encouraged them to react selectively with separate antipodal forms of chiral solutes. We chose two types of solute: those whose rate constant for reaction with e was ~ 100 times less than the collision frequency because of stereochemical, or entropy, factors (that is, no activation energy involved); and those with high asymmetry factors, such as camphor. L⁻ and DL-3-chlorobutyric acid was used as the solute of the former kind since it had $k \sim 10^6$, and the reactive site—the Cl atom—was at the asymmetric centre. We also looked for optical activity in these systems.

Just as the interaction of right-circularly polarised light is different for the D and L enantiomers of a chiral molecule, so it is supposed here that an electron confined to a right-handed helical solvent may show a different probability of reaction with D and L enantiomers: that is, it is supposed that, of the four reaction rate constants, $k_1 \neq k_2$ and $k_3 \neq k_4$, whereas $k_1 = k_3$ and $k_2 = k_4$.



where e_D and e_L are electrons solvated in right- and left-handed chiral molecules and S_D and S_L are D and L solutes.

The search for a difference in reaction rates between alike (reactions (1) and (3)) and unlike (reactions (2) and (4)) has been pursued by comparing the absolute rate constants obtained in separate experiments for the reaction of the same chiral solute in the D and L (or DL) solvent, and by scrutinising the decay curve of racemic solvated electrons when they react with a chiral solute to see if there is evidence for more than one reaction rate. (Racemic mixtures of solvated electrons would be present in a DL solvent—some environments being intrinsically symmetric, the rest being 50 : 50 mixtures of dissymmetric forms—or in a pure chiral solvent where the $+\frac{1}{2}$ and $-\frac{1}{2}$ spin states would be enantiomers.)

The experiments were carried out by pulse radiolysis using the 3-ns pulse of 600-kV electrons of a Febetron 706 accelerator. Concentrations of solvated electrons were monitored by kinetic laser photometry at 633 nm (refs 5 and 6). As chiral media we used L-2-methyl-1-butanol, D-(and DL)-2-butanol and (β) 5 M sucrose in water—for which induced chirality may be anticipated⁷. Solvated electrons in these solvents had broad structureless bands centred at ~ 700 nm. High solute concentrations ($> 10^{-2}$ M) were always used to ensure pseudo-first order decays in (e) and to limit the electron lifetime to $< 10^{-7}$ s to minimise spin flipping.

The sum (e_L) + (e_D) was determined by the measured absor-

bance at time t (A_t) in the kinetic studies on racemic solvents, such that

$$A_t = A_0\{\exp(-k_a t) + \exp(-k_b t)\}/2 \quad (5)$$

where A_0 is the absorbance at the end of the pulse ($t = 0$) and k_a and k_b are the pseudo-first order rate constants for e_L and e_D with s_L . Whenever there is non-preferential reactivity (a negative result) equation (5) reduces to a simple exponential. All the data were analysed on the basis of equation (5) through a computer fit of decay curves to give the best values of k_a and k_b .

No significant difference between k_a and k_b was found when the experiments were repeated many times using L-3-chlorobutyric acid in 2-methyl-1-butanol or L-camphor in 2-butanol. In 70% of the experimental runs involving the racemic (and achiral) solvents the analysis gave $k_a = k_b$, and the difference $|k_a - k_b|$ was not more than 10% in a significant number of cases. (In fact the decay rate during the first half life was not consistently greater than that during the second half life.) Similar results were obtained for the pure chiral solvents; k_a and k_b were found to be equivalent for at least 75% of the time.

In the search for transient optical activity we used a plane-polarised analysing light beam coupled with a crossed linear analyser before the detector. In these conditions no light reached the detector—unless the plane of polarisation was rotated on traversing the cell because of the production of unequal concentrations of e_L and e_D with dissimilar refractive indices for left- and right-circularly polarised light. No such optical rotation was found in any of these systems, even when the sensitivity of the detector was considerably increased.

In summary, we found no evidence for preferred reaction (differences in rate constant $< 10\%$) and were also unable to detect optical activity of solvated electrons in a pure chiral solvent (or in an achiral solvent containing large quantities of an unreactive chiral solute, 5 M sucrose in water) or at any stage during the decay of a racemic mixture of electrons which react with chiral solutes. Two closely related studies should be mentioned. First, tests for circular dichroic spectra of stable trapped electrons in low temperature glasses have established an upper limit of 3×10^{-3} for the asymmetry factor (g) for these solvated electron antipodes (R. May, L. D. Hayward and D.C.W., unpublished data). Second, when DL-camphor was extensively γ irradiated (using ^{60}Co γ rays, which are circularly polarised) in a chiral solvent (D-2-octanol) no preferred destruction of one enantiomer of the camphor could be detected through the development of optical activity⁸.

Apparently a rather subtle selectivity must be involved if asymmetry in nature was introduced from dissymmetry at the elementary particle level through the indirect action of the radiation.

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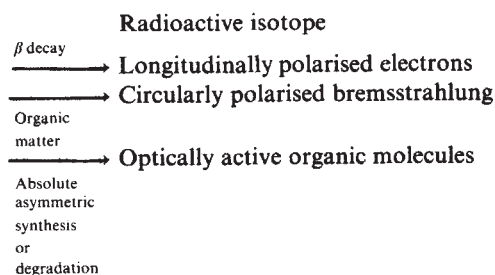
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Asymmetric degradation of DL-leucine with longitudinally polarised electrons

FOLLOWING Wu's experimental demonstration¹ of Lee and Yang's predicted violation of the parity principle during β decay² and following Goldhaber's demonstration³ of the circular polarisation of the bremsstrahlung produced by β -decay electrons, Vester and coworkers (ref. 4 and F. Vester, unpublished) proposed the following unique mechanism for the abiotic origin of optically active organic molecules



Attempts to demonstrate this mechanism experimentally^{4,5} were unsuccessful, however, until Garay's report in 1968 that D-tyrosine in dilute aqueous alkali was more decomposed (as evidenced by greater eradication of its ultraviolet absorption bands) than was L-tyrosine after 18 months exposure to 0.36 mCi of $^{90}\text{SrCl}_2$ in solution⁶. One of us has attempted⁷ to repeat Garay's experiment using DL-tyrosine in alkaline solution, looking for optical rotation after exposing the sample to a dose of 4.1×10^8 rad of β -ray bremsstrahlung in a 61,700-Ci ^{90}Sr – ^{90}Y source at Oak Ridge National Laboratory for 1.34 yr. No optical activity was noted, using optical rotary dispersion (ORD) measurements in the region 250–630 nm. At the same time⁷ Garay's experiments were extended to include other racemic amino acids, both in the solid state and in neutral, acidic or alkaline solution. In no case, after the same 1.34-yr exposure, did we notice the development of optical activity either by ORD measurements or (in the case of DL-leucine) by the gas-chromatographic determination of the enantiomeric composition of the irradiated samples. Our explanation⁷ for these negative results has been that most of the bremsstrahlung causing radiolysis of the samples was of low energy⁸ and therefore of very slight circular polarisation^{3,9}. In the hope of overcoming this difficulty, we have now undertaken similar experiments using longitudinally polarised electrons (and their bremsstrahlung) from a linear accelerator.

Our source of polarised electrons (located in the W. W. Varian Laboratories at Stanford University; see ref. 10) consists of a rotatable cathode producing a stream of 50–180-V electrons which in turn impinge on and are scattered by a beam of mercury atoms emerging from a heated mercury oven in a vacuum chamber. The scattered electrons are transversely polarised with spin up or spin down. Scattered electrons of the desired transverse polarity are selected by rotating the cathode to obtain appropriate scattering angles, and are then sent through the accelerator section of the apparatus to produce a beam of 120-keV electrons. The degree of polarisation is established by placing a Mott analyser in the beam temporarily, and the transverse polarisation is then converted to longitudinal polarisation by sending the electron beam through a 111° electrostatic beam rotator (that is, producing a relativistic 90° rotation for 120-keV electrons). The emerging longitudinally polarised electrons (natural, antiparallel, 'left-handed', or unnatural, parallel, 'right-handed') then impinge

Table 1 Irradiation of DL-leucine with longitudinally polarised electrons

Polarisation of electrons		Irradiation, (nA h)	Sample decomposition (%)	Composition of sample (corrected*)			Enantiomeric selectivity (%L-%D)
Sense	Percentage excess			%D	%L	±	
Antiparallel	~ 20	331	52.9	49.29†	50.71	0.20	1.42
Antiparallel	17-23	498	50.9	49.57‡	50.43	0.14	0.86
Parallel	11-17	795	73.9	50.40§	49.60	0.12	-0.80
Parallel	10-17	860	75.6	50.37¶	49.63	0.14	-0.74

* To analysis of DL-leucine standard.

† Four sample analyses; six DL-leucine standard analyses ($\pm 0.22\%$).

‡ Seven sample analyses; eleven DL-leucine standard analyses ($\pm 0.13\%$).

§ Ten sample analyses; twelve DL-leucine standard analyses ($\pm 0.18\%$).

¶ Thirteen sample analyses; fifteen DL-leucine standard analyses ($\pm 0.15\%$).

on the target substance held in place by Collodion on a thin sheet of aluminium foil backed by a layer of scintillation plastic (to assist focusing) and mounted on a glass window. The beam current of our 120-keV polarised electrons varied from 10 to 35 nA and the degree of polarisation from 10 to 23%.

The target material irradiated was DL-leucine (samples, 15-17 mg, $\sim 20 \text{ mg cm}^{-2}$ thick). Irradiation times were selected to produce 50-75% degradation of the leucine sample, as determined gas chromatographically by the 'enantiomeric marker' technique¹¹. The total dose was approximately 10^8 rad. Asymmetric degradation of the samples was estimated by determining the enantiomeric composition of the undecomposed leucine residue gas chromatographically, converting the irradiated sample to its N-trifluoroacetyl isopropyl ester derivative and analysing on a 150 foot $\times$ 0.02 inch capillary column coated with N-lauroyl-L-valyl-*tert*-butylamide phase¹². All irradiated samples were analysed in replicate, interspersed 'back to back' with similar replicate analyses of non-irradiated DL-leucine derivative as controls. Analyses were carried out with a Hewlett-Packard 5700A Gas Chromatograph coupled to a Hewlett-Packard 3380A Digital Electronic Integrator.

Table 1 indicates the results obtained so far in four separate irradiation experiments, two with longitudinally polarised electrons having the antiparallel left-handed spin characteristic of natural β -decay electrons¹³ and two with electrons having unnatural right-handed longitudinal polarisation. We see qualitatively that natural left-handed electrons bring about the degradation of D-leucine more extensively than that of L-leucine on irradiation of racemic DL-leucine, whereas unnatural right-handed electrons engender the asymmetric decomposition of DL-leucine in strictly the opposite sense. Unfortunately, control over the many experimental parameters of our polarised electron source—particularly the beam current and degree of net polarisation—is rather difficult, so that the data in the separate experiments of Table 1 can be compared only in a qualitative or at best merely semiquantitative fashion. It is interesting, however, that the natural left-handed electrons in our experiments produced an asymmetric degradation favouring the formation of an excess of the naturally occurring L-leucine enantiomer.

We have evaluated the analytical data of each experiment in Table 1 using the standard statistical *t*-test. Comparison of the product composition percentages in each experiment with the composition data for the simultaneously conducted replicate control analyses with N-TFA-DL-leucine isopropyl ester suggest that the composition data in each separate experiment of Table 1 differ from 50:50 at well over the 99.9% confidence level. In spite of such statistical assurances of the validity of our results, however, we have felt it additionally desirable to convince ourselves experimentally that the small differences from 50:50 which we observe in the compositions of our irradiated samples were in fact reproducibly detectable and valid using our gas chromatographic technique. Accordingly, known mixtures of D- and L-leucine of compositions approximately 49.0-51.0% and 49.5-50.5%, respectively, were prepared, converted to their N-TFA isopropyl ester derivatives, and analysed identically by gas chromatography, again interspersed 'back to back' with DL-leucine control analyses. The results of these experiments are shown in Table 2; *t*-test evaluation again indicates validity at above than the 99.9% confidence level. We thus have both experimental and statistical justification for tentative faith in the validity of the data on the irradiated samples in Table 1.

Earlier workers have been uniformly unsuccessful in inducing optical activity in racemic organic substrates by irradiations using either a variety of β -ray bremsstrahlung sources^{4,5,14} or unpolarised electron beams from various accelerators¹⁴. In addition, one of us⁷ has been unable either to repeat Garay's original type of experiment with alkaline DL-tyrosine or to observe the induction of optical activity in a number of other racemic amino acid substrates as well, in spite of using a ^{90}Sr source some 1.7×10^8 times more powerful than that used by Garay⁶. Accordingly, we believe that our present tentative observations of the asymmetric degradation of a racemic amino acid by longitudinally polarised electrons may represent the first demonstration of the potential validity of the Vester-Ulbricht mechanism for the abiotic origin of optical activity.

At present we do not know whether the asymmetric degradations in Table 1 are caused by the longitudinally polarised electrons themselves, or by their bremsstrahlung.

Table 2 Gas chromatographic analyses of D- and L-leucine mixtures of compositions approximating those of irradiated samples

	DL-leucine standard			%D	Mixture A*			%D	Mixture B*		
	%D	%L	±		%L	±	%L		±		
Known	50.00	50.00	—	49.03	50.97	—	49.51	50.49	—	—	
Observed	50.00†‡	50.00	0.08	49.05§	50.95	0.07	49.58¶	50.42	0.09	—	

* Corrected to DL-leucine standard.

† Corrected from 50.24 ± 0.08 observed.

‡ Average of eleven analyses.

§ Average of four analyses.

¶ Average of six analyses.

Relevant experiments are under way, as are attempts to enhance the extent of asymmetric degradation observed in such experiments and to extend them to encompass other amino acid substrates.

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Colour illusion and evidence for interaction between cone mechanisms

If one adapts the eye for several minutes to a yellow field of 700 cd m⁻² and then looks at a small blue target, the latter appears green for 2 or 3 s. Target and field can be provided conveniently by Wratten 12 and Illford 622 filters respectively. But the effect fails not only if the adapting field is too dim, but also if it is too bright.

This illusion may be the suprathreshold counterpart of a remarkable, but neglected, observation made in 1949 by Stiles¹: when a long-wavelength adapting field was turned off, the detection threshold for the blue-sensitive mechanism of the eye increased. We have re-examined this paradoxical loss of sensitivity to determine whether there is evidence here for an inhibitory interaction between different classes of receptor.

The existence of the phenomenon is in doubt: Das² reported results similar to those of Stiles, but there is no evidence for the anomaly in the results given by Du Croz and Rushton³. We therefore began by measuring the threshold for violet target flashes in the first seconds after a long-wavelength field had been extinguished. A yellow (580 nm) adapting field, which subtended a visual angle of 6.5°, was presented in Maxwellian view. For 3 s every 18 s the field was interrupted by a dark interval. At a particular delay after each extinction of the field a 445-nm target flash was introduced. The latter lasted 15 ms, subtended a visual angle of 1° and was delivered to the centre of a fixation array consisting of four white points arranged in a diamond. The test flash and adapting field were concentric. The experiment was under computer control and the threshold for detecting the flash on 50% of trials was measured by a double staircase procedure⁴. No data were gathered until the field had been cycling for 4 min. A block of 50 trials was devoted to the measurement of each threshold and all stimuli within a block were presented at the same delay.

Typical results are shown in Fig. 1 where the log threshold for the 445-nm test flash is plotted at various delays after the yellow field has been turned off. The broken

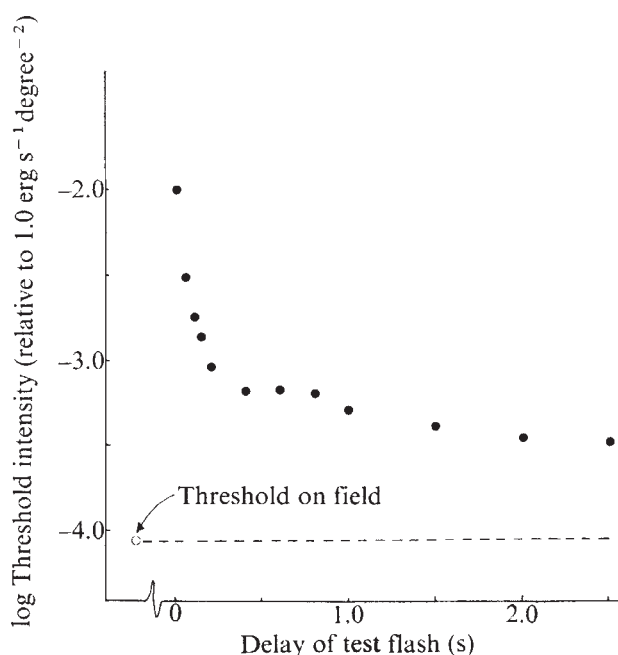


Fig. 1 Log threshold intensity for a violet test flash at various delays after the offset of a yellow field. The intensity of the field was $-1.4 \log \text{ erg s}^{-1} \text{ deg}^{-2}$. The broken line shows the threshold when the field is present. Observer, P.G.P.

line shows the threshold for the same target when the field is present. Clearly the threshold does not follow a normal dark adaptation curve, but rises immediately after the field has been turned off and after 2.5 s has not yet regained the value it had when the adapting field was present. A target that is readily visible when the background is present becomes invisible when the field is extinguished.

The suppression of the blue-sensitive mechanism is even greater than at first appears, for we found that the mechanism responsible for detection of violet flashes after the field has been extinguished has the spectral sensitivity of π_1 , Stiles' green-sensitive mechanism. The threshold for the blue-sensitive mechanism must lie above the solid points in Fig. 1. Our results so far suggest that the suppression is confined to the blue-sensitive mechanism. Therefore we provisionally call the phenomenon transient tritanopia and suggest that the suprathreshold illusion arises from the change in the relative sensitivity of green and blue cones to short-wavelength light.

In a second experiment a 580-nm field was turned off as before but was replaced immediately by a dimmer green field of 525 nm that had been empirically adjusted to have the same adaptive effect on the blue mechanism in the steady state. The threshold for 445-nm flashes was then measured at various delays after the transition between the two fields. When considering this experiment it is useful to refer to the principle of univariance⁵. A single retinal cone, or a single class of cones, is colour blind. The input to a particular cone can vary in both wavelength and intensity, but once a quantum has been absorbed all information about its wavelength is lost. In principle, therefore, it is possible to adjust the intensities of two adapting fields until they are indistinguishable to the blue cones. According to the classical model of Stiles the adaptive states of the cone mechanisms are independent: that is, the sensitivity of an individual mechanism depends only on the number of quanta absorbed from an adapting field by that particular mechanism; and thus two adapting fields that raise the threshold equally for the short-wavelength target must be producing an equal quantum catch in the blue cones. If the sensitivities of the mechanisms are indeed independent in the steady state and if they remain independent in transitional states then transient tritanopia must

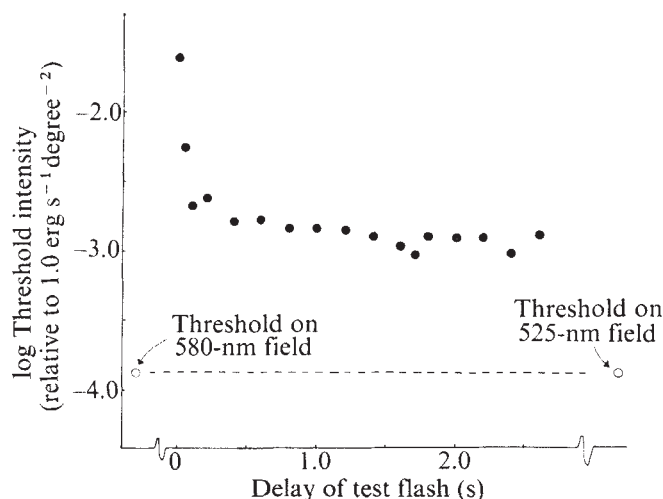


Fig. 2 Solid circles show the threshold for a 445-nm target at various delays after the transition between 580-nm and 525-nm fields. The two fields were adjusted to raise the threshold by the same amount in the steady state: the two open circles, each based on ten determinations, show the empirical thresholds obtained in the steady state. Observer, J.D.M.

be a private matter among the blue cones and there should be no disturbance of sensitivity when we switch from the yellow to the green field. If, however, the threshold still rises after the transition, then there must be an interaction between mechanisms: the interaction may occur in the steady state, so that fields that raise the 445-nm threshold equally do not produce equal absorption in the blue cones (such a suggestion has been made, see for example, ref. 6); alternatively or in addition, a recovering long-wavelength mechanism may inhibit, or otherwise suppress, the blue cones during early dark adaptation, for the number of quanta absorbed by the long-wavelength mechanisms will fall as the transition is made between yellow and green fields that have the same adaptive effect on the blue cones.

The threshold for 445-nm flashes was measured on a steady yellow field of $-1.36 \log \text{ erg s}^{-1} \text{ degree}^{-2}$ and a steady green field was then adjusted until it raised the threshold for the violet flash to the same level. In a final check the thresholds on the two steady adapting fields were repeatedly measured in an alternating sequence. The two mean thresholds, each based on ten determinations, were within 0.01 $\log_{10}$ units of each other and were approximately 0.5 $\log$ units above the value for the dark-adapted eye. Auxiliary measurements of increment threshold functions showed that detection was mediated by the blue-sensitive receptor mechanism that was termed π_1 by Stiles⁷. Figure 2 shows that transient tritanopia still occurs when the transition is made between two fields that have the same adaptive effect in the steady state: for several seconds after the substitution of the green field the threshold remains almost 1.0 $\log_{10}$ units above its value on either of the steady fields.

We conclude that the blue-sensitive receptors are subject to inhibition from a mechanism with a different spectral sensitivity. Whether the interaction is confined to transitional conditions remains to be determined. Stiles distinguished three short-wavelength mechanisms, π_1 , π_2 , and π_3 : it is possible that we see in transient tritanopia an exaggeration of an inhibition that marks the difference between these mechanisms in the steady state.

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Learning in the absence of forebrain noradrenaline

CONSIDERABLE attention has been given to suggestions that forebrain noradrenaline (NA) plays a central role in the associative learning process^{1,2}. For example, Kety² has proposed a general noradrenergic theory of learning in which he hypothesises that NA release associated with reinforcement facilitates the formation of learned associations. Crow³ subsequently suggested that the dorsal NA bundle which extensively innervates the cortex could be the crucial pathway involved. In support of this hypothesis are findings that learning on shock avoidance⁴ and appetitive^{5–7} tasks is impaired after widespread loss of forebrain NA and dopamine. More specifically, Anlezark *et al.*⁸ found that bilateral electrolytic lesions to the origins of the dorsal NA bundle in the locus coeruleus impaired the acquisition of runway behaviour. Cortical forebrain NA loss was reduced to 69% of control levels in their study. We have used 6-hydroxydopamine (6-OHDA) treatments to achieve virtually total forebrain NA loss and have found no impairment on the acquisition of runway behaviour. The NA-depleted animals, were however, slow to inhibit running during extinction, suggesting that although the dorsal NA system is not involved in the acquisition of reinforced responses, it is important for the response to non-reward.

Fig. 1 Acquisition of running in alleyway. Subjects were deprived to 90% of free feeding weight. a, NPT; b, DBT. 6-OHDA was dissolved in 0.9% NaCl containing ascorbic acid (1.0 mg ml⁻¹). Infusion rate was 1 $\mu\text{l min}^{-1}$ in the DBT preparation. Appropriate sham-operated and injected controls were prepared for both groups. Time to run from start to goal box plotted against day of training. Time values are means of the five trials on each day. □, Controls; ■, treated.

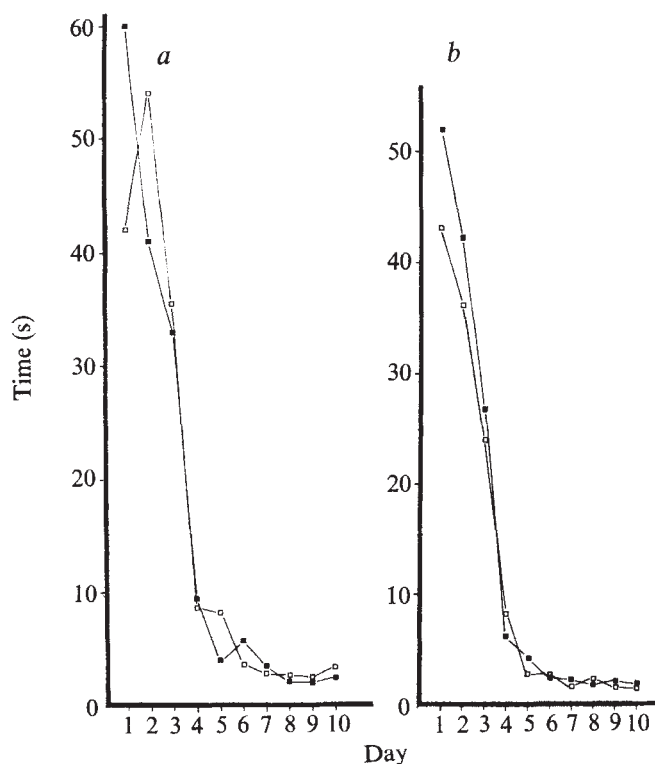


Table 1 Regional concentrations of NA and dopamine (pg per g tissue) in treated and control animals on the two depletion techniques

	NPT			DBT		
	Controls (n = 9)	Treated (n = 9)	t Statistic	Controls (n = 5)	Treated (n = 5)	t Statistic
NA						
Hippocampus	0.415 ± 0.13	0.010 ± 0.007	9.34	0.233 ± 0.069	0.0 ± 0.0	7.58
Hypothalamus	1.10 ± 0.061	1.34 ± 0.094	6.43	0.708 ± 0.365	0.118 ± 0.141	3.38
Cortex	0.205 ± 0.013	0.017 ± 0.002	42.9	0.174 ± 0.068	0.001 ± 0.002	5.71
Rest of brain	0.365 ± 0.040	0.508 ± 0.035	8.07	0.218 ± 0.049	0.208 ± 0.027	0.41
Dopamine						
Hypothalamus	0.43 ± 0.13	0.32 ± 0.09	2.08	0.079 ± 0.167	0.151 ± 0.213	0.60
Striatum	4.71 ± 0.30	4.43 ± 0.32	1.92	5.45 ± 1.82	7.03 ± 2.83	1.05

Values are followed by s. d.

Two different 6-OHDA treatments were used, both of which result in adult rats with virtually total loss of NA from the cortex and hippocampus. In the systemically treated neonate preparation (NPT), 10 albino Wistar rats were injected intraperitoneally on days 1, 3, 5, 7, 9, 11 and 13 after birth with 6-OHDA hydrobromide (100 mg kg⁻¹). Behavioural testing was begun at 3 months of age. In the second technique (DBT), 10 male albino Wistar rats, 2.5 months old received bilateral injections of 6-OHDA into the dorsal bundle using stereotaxic coordinates derived from König and Klippel⁹. Behavioural testing started 2 weeks after the operation. An L-shaped runway was used, of stem length 100 cm and arm length 30 cm; it was painted matt black throughout. The subject was placed in the start box and the door raised when the subject was orientating towards it. The running time in the stem was measured using electronic timers triggered by photocell beam interruption. The beams were located immediately beyond the start box and immediately before the goal box. On entering the goal box the subject was presented with a shallow glass food cup containing four food pellets (45 mg each, P. J. Noyes Company, Lancaster, New Hampshire). At the end of a trial the subject was constrained in the goal box for 10 s; after about 15 s in an inter-trial cage the subject was returned to the start box for the next trial. Five trials a day were carried out until running speeds had reached a plateau (three successive days being within 10% of each other). All animals had reached this plateau within 10 d. Extinction was begun on day 11 and the procedures were identical except that no food cup or food were present in the goal box. Extinction trials continued until the subject's latency was ≥ 10 times the latency on trial 1 of extinction.

No difference in the number of trials to criterion was found between either treated group and its control group during the acquisition period of L-maze running (Fig. 1) (analysis of variance—NPT: $F = 0.058$, d.f. = 1,18, $P \gg 0.05$; DBT: $F = 0.46$, d.f. = 1,18, $P \gg 0.05$). Furthermore, considering speed of running there was no difference between treated and control groups on trial 1 of acquisition (NPT: t statistic = 0.29, d.f. = 16, $P \gg 0.05$; DBT: t statistic = 1.21, d.f. = 21, $P > 0.05$) confirming that neither of the two NA depletion techniques had produced a nonspecific motor impairment. Both treated and control groups started extinction after the last acquisition trial running at the same speed (trial 1 extinction, NPT, t statistic = 1.09, d.f. = 16, $P > 0.05$; DBT: t statistic = 0.095, d.f. = 18, $P > 0.05$). On extinction, however, both treated groups showed maintained rapid running and resistance to extinction (Fig. 2) (number of trials to extinction criterion, Mann-Whitney U Test—NPT: $U = 3.50$, $P < 0.002$; DBT: $U = 2.00$, $P < 0.001$).

At the end of testing the nine surviving NPT rats and five DBT rats together with their controls were decapitated, and their brains rapidly dissected on ice and subdivided into brain stem, striatum, hypothalamus, cortex and hippocampus. These brain areas were homogenised in perchloric acid and assayed for regional concentrations of NA and dopamine according to Cuello *et al.*¹⁰. The results confirmed that both treatments

had produced severe depletion of NA in the cortex and hippocampus, leaving brain dopamine intact (Table 1).

These results show that with two different selective chemical techniques which result in severe loss of hippocampal and cortical NA, acquisition of L-maze running for food reward was not impaired. This contrasts with the impairment reported⁸ to occur after surgical lesions to the locus coeruleus which also deplete forebrain NA, albeit to a lesser degree. We suggest that in the case of surgical lesions the impairment may stem from effects of the lesion other than the observed depletion of NA.

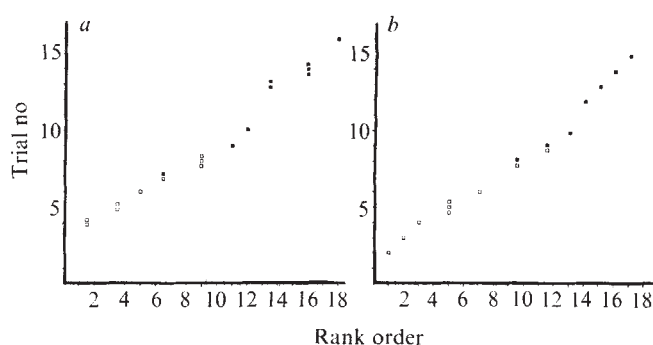


Fig. 2 Extinction of alleyway running. *a*, NPT; *b*, DBT. Trial number on which extinction criterion (see text) was reached plotted against overall rank order of extinction over both groups for each animal. Tied ranks represented by mean value of ranks and with multiple points at that value. □, Controls; ■, treated.

The perseveration in extinction after both treatments reported in the present experiments indicates, however, that forebrain NA depletion produces marked changes in behaviour. The dorsal bundle lesioned animals also show resistance to extinction of bar pressing after training on a continuous reinforcement schedule, and in perseveration to the negative stimulus in a successive light-dark discrimination task (S.T.M. and S.D.I., unpublished). These are all situations in which behaviour is required to change in response to non-reward. Gray¹¹ has proposed that a particular frequency of θ electrical activity in the hippocampus reflects the coding of non-reward. This frequency (7.7 Hz) has the lowest threshold of elicitation by electrical stimulation of the pacemaker cells for the θ rhythm located in the medial septal area. This threshold is selectively raised by anti-anxiety drugs. The finding of Gray *et al.*¹² that lesions of the dorsal NA bundle act in the same way as the anti-anxiety drugs, in selectively raising the threshold for 7.7-Hz septal-induced hippocampal electrical activity, is further support for a role of this NA system in processes concerned with non-reward. Further research to elucidate these effects is planned and it is hoped to develop 6-OHDA lesion techniques which will selectively deplete either cortical or hippocampal noradrenaline.

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Effect of minor tranquillisers on hippocampal θ rhythm mimicked by depletion of forebrain noradrenaline

THERE are similarities between the behavioural effects of minor tranquillising drugs and those of lesions to the septal area and hippocampus¹. Since the septal area contains the pacemaker cells for the hippocampal theta rhythm², a parsimonious hypothesis to account for these common effects is that tranquillisers affect behaviour by altering septal control of this rhythm. Sodium amobarbitone (SA) has been shown to block the behavioural effects of frustrative non-reward¹, as do septal³ and hippocampal⁴ lesions. Rats exposed to non-reward in the runway show a characteristic hippocampal θ response at 7.7 Hz (ref. 1) (the θ frequency range in the rat is about 6-12 Hz). Higher and lower frequencies occur during forms of behaviour which are unaffected by the drug. It has therefore been proposed¹ that the behavioural effects of SA arise by virtue of an impairment of septal control of the hippocampal θ rhythm specifically in a frequency band centred on 7.7 Hz. In support of this, it has been demonstrated⁵ that this drug raises the threshold for septal driving of the hippocampal θ rhythm selectively at 7.7 Hz, frequencies both above and below this value being minimally affected. Here we show that this kind of frequency-specific effect on septal driving of the hippocampal θ rhythm is also produced by other drugs with similar effects on behaviour¹ (ethanol, chlordiazepoxide and Δ^9 -tetrahydrocannabinol (Δ^9 -THC)), by drugs which impair noradrenergic neural transmission, and by destruction of the dorsal ascending noradrenergic bundle by intracerebral injection of 6-hydroxydopamine (6-OHDA)⁶.

The techniques used for electrode implantation and for stimulation and recording have been described previously^{1,7}. The subjects were free-moving male albino rats with a bipolar stimulating electrode chronically implanted in the medial septal nucleus and a bipolar recording electrode in the dorso-medial subiculum. (J. N. P. Rawlins has shown that the θ rhythm recorded here is identical in phase and frequency to that recorded in CA1.) In this preparation it is possible to drive the hippocampal theta rhythm by septal stimulation, the frequency of the driven wave corresponding exactly to the frequency of the applied stimulation. To obtain a driving threshold at a particular frequency of septal stimulation, a Tektronix 502A dual-beam oscilloscope was set to be triggered by the stimulus pulse

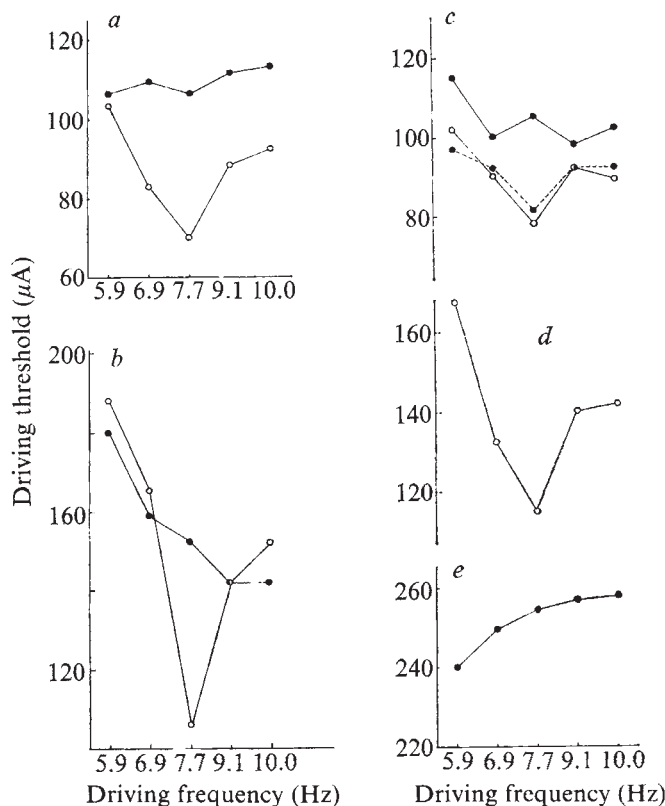


Fig. 1 Effects of various treatments on threshold for septal driving of hippocampal θ rhythm as a function of stimulation frequency (θ -driving curve). *a*, *b*, and *c*, Average results obtained from a group of male rats before and after drug treatment. Control injections consisted of appropriate vehicle alone. *a*, Chlordiazepoxide HCl, $n=4$, 5 mg kg⁻¹ intraperitoneally (i.p.) in 1 ml kg⁻¹ saline 30 min before test. *b*, Δ^9 -THC, $n=4$, 0.5 mg kg⁻¹ i.p. in 1 ml kg⁻¹ Tween 80-saline solution 20 min before test. *c*, α -MPT, $n=4$, 100 mg kg⁻¹ i.p. in 1 ml kg⁻¹ saline followed 7.25 h later by 30 mg kg⁻¹ *dl*-threo-3,4-dihydroxyphenylserine (DOPS) i.p. suspended in 1 ml kg⁻¹ saline; readings were taken 6.5 h after α -MPT and 70 min after DOPS. *d*, Average of three rats given control injection of saline-ascorbic acid vehicle in dorsal ascending noradrenergic bundle; *e*, average of three rats injected with 6-OHDA in dorsal bundle. *a* and *b*, ●, Drug; ○, control; *c*, ●, α -MPT; ---, DOPS; ○, control.

(0.5 ms d.c., square wave, from an isolated constant-current source) and the stimulating current was gradually increased in intensity until the hippocampal EEG (displayed on the second beam of the oscilloscope after amplification by a Grass 7P5B pre-amplifier) showed waves which remained in phase with the stimulus. Five stimulating frequencies were used (see Fig. 1) and five readings were taken in random order at each of these frequencies over a 30-min experimental session. The readings were then averaged to construct a ' θ -driving' curve (Fig. 1). The stability of the readings obtained in this way is good, as is agreement between different experimenters. In the undrugged male rat there is a minimum or dip located at 7.7 Hz (Fig. 1). In a control series of 26 male rats, 24 (92%) displayed such a dip.

We have investigated, in a total of 19 rats, the changes produced in the θ -driving curve by injections of SA, ethanol, chlordiazepoxide and Δ^9 -THC. The drug parameters used are known to be behaviourally effective in relevant situations^{1,8}. All four produced their largest effect at 7.7 Hz, thus eliminating the dip in the curve at this frequency. The results with chlordiazepoxide and Δ^9 -THC are illustrated in Fig. 1*a* and *b*. The results with 15 mg kg⁻¹ SA and 1,200 mg kg⁻¹ ethanol closely resembled those obtained with the other two drugs, as well as those previously obtained with 20 mg kg⁻¹ SA (ref. 5). The data from the four drug treatments were logarithmically transformed (to eliminate inhomogeneity of variance) and submitted jointly to analyses of variance for the effects of drug

Table 1 Effect of 6-hydroxydopamine injection into dorsal ascending noradrenergic bundle on regional levels of noradrenaline and dopamine

	Vehicle injected ($\mu\text{g g}^{-1}$)	6-OHDA injected ($\mu\text{g g}^{-1}$)	<i>t</i>	<i>P</i>
Hippocampus NA	0.30 $\pm$ 0.04	0.01 $\pm$ 0.01	9.67	<0.001
Hypothalamus NA	1.97 $\pm$ 0.75	0.97 $\pm$ 0.28	1.71	NS
Hypothalamus DA	0.54 $\pm$ 0.13	0.53 $\pm$ 0.09	0.06	NS
Striatum DA	10.22 $\pm$ 3.07	8.49 $\pm$ 1.13	0.75	NS
Cortex NA	0.39 $\pm$ 0.16	0.04 $\pm$ 0.03	3.05	<0.025
Septum NA	0.82 $\pm$ 0.34	0.31 $\pm$ 0.12	1.99	NS

Values shown are means ($n = 3$) $\pm$ standard error.
NS, Not significant.

(that is, the four separate drugs), treatment (that is, drug against placebo) and stimulation frequency. There was a significant interaction between treatment and stimulation frequency ($F = 4.4$; d.f. = 4 and 135; $P < 0.01$) but the drug $\times$ treatment $\times$ frequency interaction was small ($F < 1$), both tested against the within-subject residual variance. Thus, the four drugs had essentially the same frequency-specific effect: the threshold at 7.7 Hz was significantly lower than at any other frequency in the combined control condition but not after treatment with any of the drugs.

We have tried to mimic the characteristic change produced in the θ -driving curve by the minor tranquillisers by using other drugs with better understood neurochemical effects. We have been unable to do this with cholinergic or anticholinergic drugs (0.05 or 0.5 mg kg⁻¹ physostigmine, 0.5 mg kg⁻¹ scopolamine); and Stewart and Gray (unpublished) failed to do so by blocking the synthesis of 5-hydroxytryptamine (with 300 mg kg⁻¹ *p*-chlorophenylalanine). We have, however, obtained evidence of a function for noradrenergic neurones. We used α -methyl-*p*-tyrosine (α -MPT), which blocks the synthesis of dopamine (DA) and noradrenaline (NA)⁹, followed by dihydroxyphenylserine (DOPS), an artificial precursor of NA but not of DA¹⁰. α -MPT acted on the θ -driving curve as did the tranquillisers and this action was reversed by DOPS (Fig. 1c). Analyses of variance showed a significant treatment $\times$ frequency interaction ($F = 6.3$; d.f. = 4 and 12; $P < 0.01$) for α -MPT against control, but no significant difference from control after further treatment with DOPS ($F < 1$).

These results indicate a noradrenergic pathway which facilitates septal driving of the hippocampal θ rhythm maximally at a frequency of 7.7 Hz. The pathway most likely to produce this effect is the dorsal ascending noradrenergic bundle originating in the locus coeruleus, which innervates the neocortex, medial septal area and hippocampus^{6,11}. We used intracranial injections of 6-OHDA to destroy this pathway. Eight rats were anaesthetised and placed in a stereotaxic instrument. A needle, aimed at the dorsal bundle (Plate I of ref. 6), was lowered and 4 or 8 μg of 6-OHDA in 4 μl of cooled saline containing ascorbic acid (1 μg μl^{-1}) was injected bilaterally at a rate of 1 $\mu\text{l min}^{-1}$. Bipolar subicular recording electrodes and a septal stimulating electrode were then implanted bilaterally. Of the eight rats so treated, three showed θ driving (about our usual success rate in unlesioned rats). None displayed a 7.7-Hz dip in the θ -driving curve from either hemisphere (Fig. 1e). Comparison with the untreated control series shows this result to be highly significant ($\chi^2 = 16.2$, d.f. = 1, $P < 0.001$). In three control rats, given an injection of the saline-ascorbic acid vehicle in the dorsal bundle, the θ -driving curve was normal (Fig. 1d).

Regional levels of NA and DA were determined in the 6-OHDA- and vehicle-injected rats. The rats were stunned and decapitated. The brains were dissected on an ice-cold glass plate¹², and NA and DA were assayed by a radioenzymatic method¹³. The results (Table 1) showed that the lesion produced a large (97%) decrease in hippocampal NA, a smaller (non-significant) decrease in hypothalamic NA and no change in hypothalamic or striatal DA. Thus the change produced in the θ -driving curves of the experimental animals probably re-

flects the loss of the noradrenergic afferents to the hippocampus and/or septal area from the locus coeruleus.

The results of these experiments suggest that the dorsal noradrenergic bundle exercises a frequency-specific facilitation of the septally-controlled hippocampal θ rhythm, and that the minor tranquillisers impair this noradrenergic input to the hippocampus (as previously indicated by biochemical observations¹⁴). This impairment may, in part, underlie the behavioural effects of the minor tranquillisers. If so, destruction of the dorsal bundles should produce the same effects on behaviour as injection of a minor tranquilliser. Such effects have been found in situations involving frustrative non-reward by Mason and Iversen¹⁵.

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Coupling of lithium to sodium transport in human red cells

THE administration of lithium salts is often used in the treatment of affective psychoses¹. Mendels *et al.*²⁻⁴ and others⁵ have measured the concentrations of lithium in blood plasma and red cells of patients and normal individuals receiving such salts. They have argued that the lithium concentrations in red cells can be correlated with the clinical status of these people⁴ and, therefore, can possibly be related to the pathogenesis of the mental disorders. They found that the concentration of lithium in red cell water is about one-third of that in the blood plasma during chronic administration of LiCl (ref. 4). This fact is surprising since it would suggest that Li⁺ is actively transported by the red cell membrane. The steady-state value of the ratio

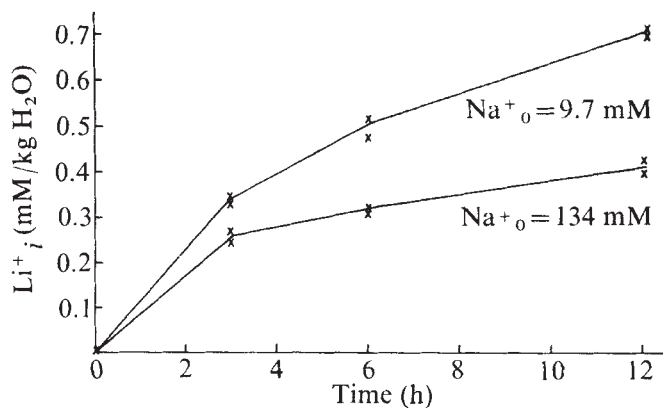


Fig. 1 Typical results from one (experiment 86) of three identical experiments each carried out in duplicate. Fresh human red cells, washed in isotonic NaCl, and incubated (5% v/v) for 12 h at 37 °C in a medium containing LiCl (1.5 mM), KCl (5 mM), glycyl glycine (25 mosM, pH 7.3), glucose (5 mM), adenosine (5 mM), ouabain (10^{-4} M) and either NaCl (140 mM) (high Na^+ medium) or NaCl (10 mM) plus MgCl_2 (85 mM) (low Na^+ medium). There was no significant change in the composition of the medium during the incubation.

Change in cell composition during experiment						
Time (h)	Na^+_o (mM)	Li^+_o (mM)	H_2O_i (w/w)	Li^+_i mM per l original cells	Na^+_i	K^+_i
0	134	1.5	0.658	0.00	7.2	75
3	134	1.5		0.17	15	73
6	134	1.5		0.22	17	69
12	134	1.5	0.642	0.27	19	69
0	9.8	1.5		0.00	7.2	75
3	9.8	1.5		0.22	7.2	76
6	9.8	1.5		0.32	6.4	76
12	9.8	1.5	0.642	0.46	6.6	72

of internal to external concentration for a monovalent cation which is not actively transported should be about 1.2 (ref. 6). Previous observations in red cells⁷ and squid axons⁸ suggested that Li^+ is not transported by the Na-K pump. Furthermore, the net efflux of Li^+ into Li^+ -free medium was unaffected by the presence of ouabain⁴. The experiments described here were designed to clarify the mechanisms of this seemingly ouabain-insensitive 'active' transport of lithium ions in human red cells.

The steady-state concentration ratio for Li^+ is about twice as high in low potassium (LK) as in high potassium (HK) sheep red cells⁴, and this is consistent with the hypothesis that Li^+ transport in this system occurs by an ouabain-insensitive process of Li^+ - Na^+ exchange diffusion⁶. By this process, Li^+ would move through the red cell membrane only in exchange for some other cation which might be Li^+ or Na^+ . If this is the only, or the major, mechanism of Li^+ transport, the steady-state distributions of Li^+ and Na^+ would be similar. If Na^+ is also transported by the ouabain-sensitive Na^+ - K^+ pump and the ouabain-insensitive but voltage-dependent leak pathways which maintain the electrochemical potential of Na^+ lower inside than outside of the red cells, then the Na^+ - Li^+ exchange process could maintain a similar asymmetry in the distribution of Li^+ . In this system, the energy for the 'active' transport of Li^+ would be provided by dissipation of the electrochemical potential difference for Na^+ . Our results are consistent with this hypothesis.

We measured the effect of external Na^+ on the uptake and extrusion of Li^+ by red cells freshly drawn from normal human donors. The cells were washed free of plasma with isotonic NaCl, suspended in an appropriate medium (5% v/v) and incubated (37 °C, pH 7.4). All media contained LiCl (~ 1.5 mM), KCl (5 mM), glycyl glycine (25 mosM, pH 7.4) and ouabain (10^{-4} M). Samples of these cell suspensions taken at intervals were centrifuged, the cells washed in ice-cold, isotonic MgCl_2 solutions and lysed⁹. The lysates as well as samples of the extracellular medium, were analysed for Na^+ , K^+ and Li^+ by atomic absorption spectrophotometry and for haemoglobin by spectrophotometry⁹. Water content of the cells was estimated by measuring wet and dry weights.

The results of the first type of experiment are shown in Fig. 1. Cells were incubated in media containing either ~ 140 mM NaCl (high Na^+ medium) or ~ 10 mM NaCl (low Na^+ medium, MgCl_2 replaced NaCl to maintain isotonicity). The rate of Li^+ accumulation was substantially greater in cells exposed to the low Na^+ medium. External Na^+ inhibits Li^+ uptake.

Figure 2 describes the results of the second type of experiment. Cells were incubated for 12 h in a low Na^+ medium containing 1.5 mM LiCl. These Li^+ -loaded cells were then resuspended in either high or low Na^+ medium. The cells exposed to the high Na^+ medium promptly extruded Li^+ against the electrochemical potential gradient, whereas the cells in the low Na^+ medium

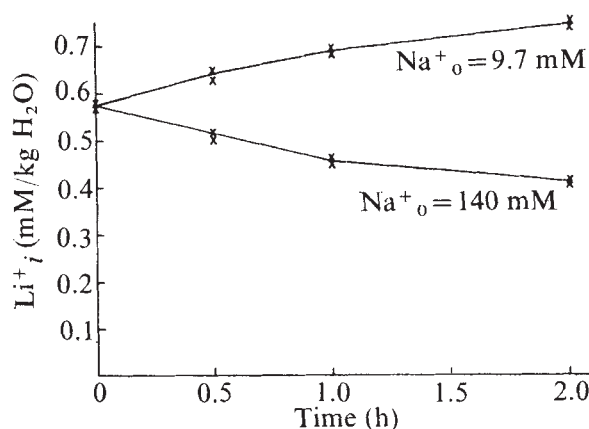


Fig. 2 Typical results from one (experiment 90) of three identical experiments, each performed in duplicate. Fresh human red cells washed in isotonic NaCl and incubated (5% v/v) for 12 h at 37 °C in low Na^+ medium were resuspended in either low Na^+ or high Na^+ medium (as defined in Fig. 1) and again incubated at 37 °C. The composition of the medium did not change with time during this second incubation, while the composition of the cells changed as shown in the accompanying table.

Change in cell composition during experiment						
Time (h)	Na^+_o (mM)	Li^+_o (mM)	H_2O_i (w/w)	Li^+_i mM per l original cells	Na^+_i	K^+_i
0	140	1.6	0.649	0.37	6.4	91
0.5	140	1.6		0.32	9.2	91
1.0	140	1.6		0.29	9.4	89
2.0	140	1.6	0.629	0.26	9.6	86
0	9.7	1.2	0.649	0.37	6.4	91
0.5	9.7	1.2		0.41	6.4	92
1.0	9.7	1.2		0.47	6.5	90
2.0	9.7	1.2	0.641	0.48	6.4	89

continued to gain Li^+ . Li^+ is 'actively' transported out of the cells in the presence of an electrochemical potential difference and for Na^+ and ouabain.

Table 1 shows the result of an experiment in which human red cells were loaded with Na^+ and Li^+ by the Nystatin method. When these cells were incubated in media identical to those described in Fig. 1 (except that Li^+_0 was 1.0 instead of 1.5 mM), Li^+ accumulation against an electrochemical potential gradient occurred in cells exposed to a low Na^+ medium but not to a high Na^+ medium. Net uphill transport of Li^+ occurs in either direction across the human red cell membranes depending on the direction of the Na^+ electrochemical potential gradient.

Table 1 Incubation of human red cells

Time (h)	Na^+_0 (mM)	Li^+_0 (mM)	H_2O_1 (w/w)	Li_1 mM per l original cells	Na_1
0			0.631	0.91	112
0.5				0.86	106
1.0				0.85	104
2.0	130	1.06	0.637	0.85	112
0			0.631	0.91	112
0.5				1.02	97
1.0				1.22	94
2.0	10	1.02	0.644	1.30	92

These results agree with the hypothesis that ion exchange counter flow is the major mechanism of net uphill Li^+ transport in human red cells. Further confirmation will require demonstration of net uphill Na^+ transport driven by an oppositely-directed Li^+ electrochemical potential gradient. The data support the conclusion that an electrochemical potential gradient for Na^+ is a sufficient condition for such 'active' transport of Li^+ . The properties and physiological significance of this Na^+-Li^+ transport system can now be studied. The dependence of the exchange process on external and internal concentrations of Na^+ and Li^+ must be defined. Another line of investigation will be to explore the relationship between the Na^+-Li^+ exchange system and the $\text{Na}^+-\text{Ca}^{2+}$ exchange system known to operate in nerve membranes¹⁰. Such a relation could be important in the therapeutic action of Li^+ in view of the known function of Ca^{2+} in the release of neurotransmitters from synaptic vesicles¹¹. The existence of ion exchange systems when one partner (for example, Na^+) is also actively transported permits the coupling of the other partner (for example, Li^+) to the active transport process even though the latter is not itself pumped.

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Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity

ATAXIA telangiectasia (AT) is an autosomal recessive defect in man showing among the clinical features¹: cerebellar ataxia, telangiectasia, IgA deficiency, an enhanced frequency of malignancy and an enhanced level of spontaneous chromosome instability². There have also been reports of increased sensitivity to X rays after radiotherapy³⁻⁵ and increased chromosome aberrations induced by ionising radiation in leukocyte cultures from AT patients^{6,7}. We

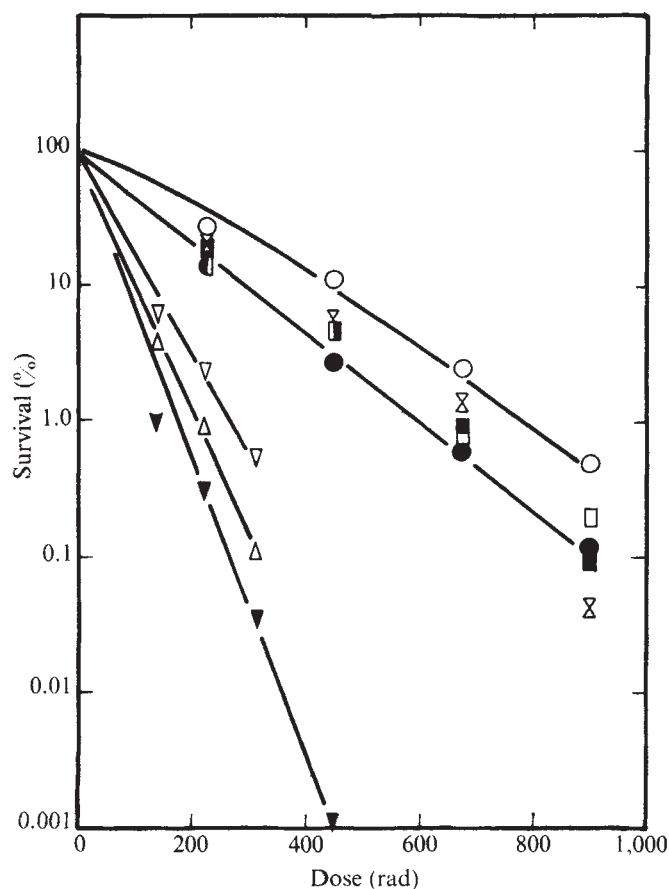


Fig. 1 γ -Ray response of cultured human fibroblasts. Lines fitted by eye. $\circ$, 1BR, normal, age 25 yr, male, 7 experiments; $\bullet$, 2BI, normal, 25 yr, male, 8 experiments; $\square$, BCNS1BI, 38 yr, male, 5 experiments; double triangles, BCNS2BI, 74 yr, female, 4 experiments; $\blacksquare$, BCNS3BI, 14 yr, male, 5 experiments; $\triangle$, AT1BI, 7 yr, male, 3 experiments; ∇ , AT3BI, 4 yr, male, 2 experiments; $\blacktriangledown$, AT4BI, 6 yr, male, 1 experiment. Cultures were maintained on Eagles' minimal essential medium plus 15% foetal calf serum (Flow Laboratories) in glass or plastic vessels. Cells were detached from the surface with 0.25% trypsin (Sigma) in Dulbecco's A saline and resuspended in appropriate concentrations in complete medium before irradiation (in air) by a cobalt-60 source with a dose rate of 2.7 krad min^{-1} . A tenfold dilution in unirradiated medium containing 15% foetal calf serum (Sera Services) or calf serum (Flow Laboratories) to remove any influence of radiation on the components of the medium always preceded plating on 9-cm (Nunc) dishes. Two plating protocols have been practised. (1) The thin feeder layer technique¹³: cultures were kept in sealed polystyrene boxes with a 5% CO_2 in air mixture in an anhydric incubator for 14-16 d and with a medium change after 7 d. (2) Cells were plated without feeder layer and kept in CO_2 incubators with medium changes every 3 d for 14-16 d. At the end of the growth period colonies were stained with 1% methylene blue. A colony containing 50 cells was counted as a survivor. The mean cloning efficiency in this series of experiments was 28%. While we have noted a correlation between cloning efficiency and number of cell doublings in a culture, particularly in early subcultures, there was no indication of a correlation between cloning efficiency and cell survival.

report here cell survival experiments which indicate that the clinically observed enhanced sensitivity of AT patients to ionising radiation is manifest at the cellular level.

Figure 1 illustrates the response to γ rays of fibroblast cell lines from the skin of two normal donors, three AT patients and three patients with the basal cell naevus syndrome (BCNS). BCNS has, like AT, shown indications of an association between this condition and enhanced spontaneous and X-ray-induced chromosome aberrations⁴. The response of the normal cell strains falls, in our experience, within the range shown by the two individuals whose survival curves are shown in Fig. 1. (So far we have examined cell strains from eight individuals.) Clearly the BCNS cells fall within the normal range. The cells from the three AT patients, however, show greatly enhanced sensitivity. A similarly enhanced sensitivity was seen after irradiation of AT4BI fibroblasts with 300 keV X rays, when compared with two further control cell strains. It is interesting that patient AT1BI was the same patient in whom Cunliffe *et al.*⁵ showed radiosensitivity *in vivo*.

In preliminary attempts to characterise the biochemical basis of the enhanced sensitivity we have compared the production and repair of γ -ray induced single and double-strand breaks in the DNA of normal and AT cells. Like other

mammalian cells, human fibroblasts can rejoin single-strand breaks, most being rejoined within 1 h of the post-irradiation incubation, as demonstrated by sedimentation patterns obtained from alkaline sucrose gradients (ref. 9 and confirmed in our experiments). The rates of rejoining of single-strand breaks were very similar in fibroblasts from a normal donor and from an AT patient (Fig. 2a and b).

Using neutral sucrose gradients, we found in earlier investigations that DNA from unirradiated mouse cells formed a rapidly sedimenting peak (RSP). Ionising radiation released free DNA molecules from the RSP and higher doses of radiation decreased the molecular weight of the free DNA¹⁰. We suggested that these changes in sedimentation behaviour in non-denaturing conditions might be a consequence of the introduction of double-strand breaks into the DNA. Using similar experimental conditions we have now observed comparable behaviour with human fibroblasts. A dose of 30 krad released most of the DNA from the RSP. During post-irradiation incubation the RSP was reformed (Fig. 2c). If the release of DNA from the RSP is indeed a consequence of the introduction of double-strand breaks, then reformation of the RSP, which represents a return to the sedimentation behaviour characteristic of that from unirradiated cells, is probably a manifestation

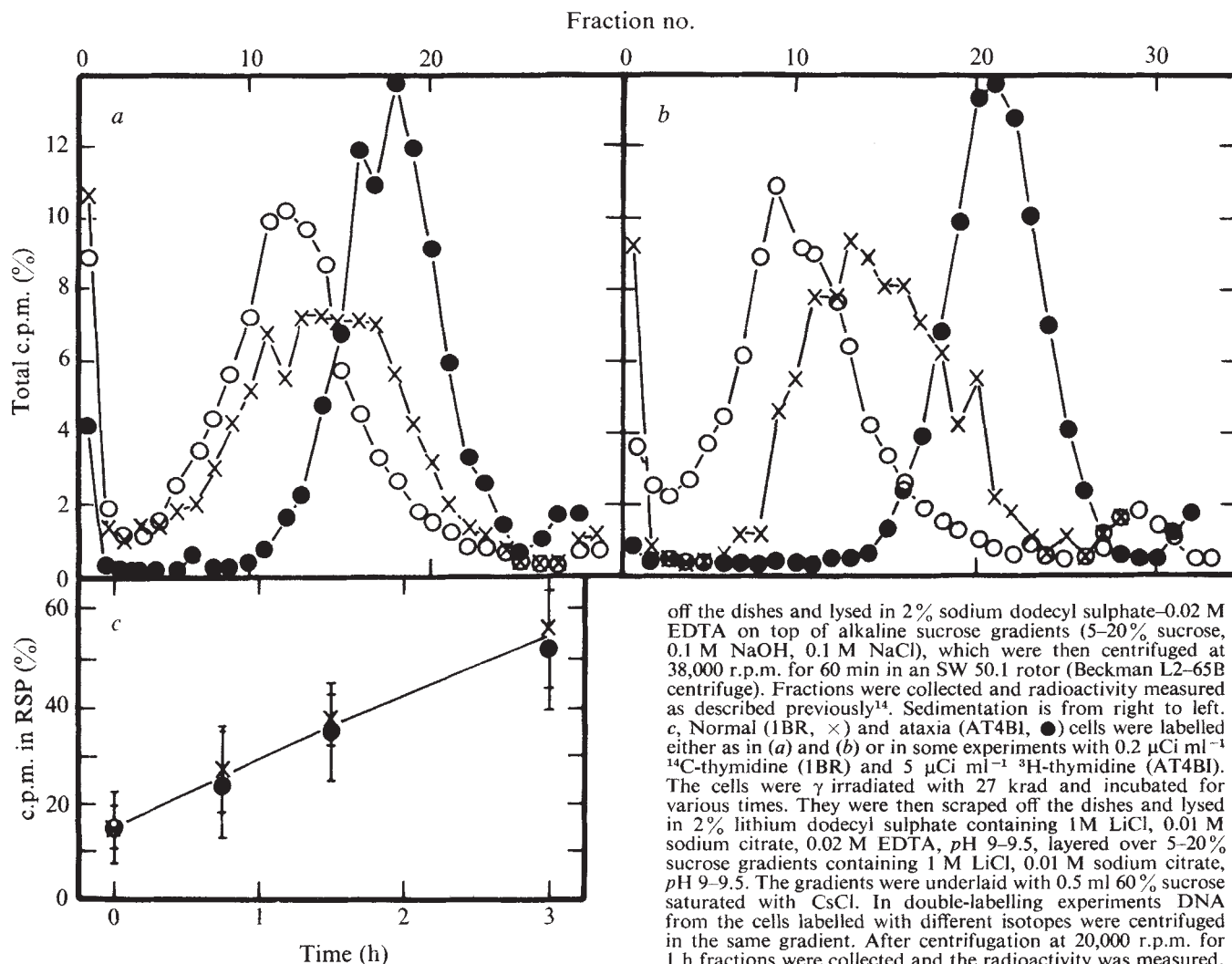


Fig. 2 Rejoining of strand breaks in normal and ataxia cells. 10^6 cells of 2BI (a) or AT1BI (b) were incubated in 5-cm Petri dishes. On the next day the cells were labelled with $1.5\text{--}5\text{ }\mu\text{Ci ml}^{-1}$ ^3H -thymidine (5 Ci mmol^{-1}), for 16–24 h. After removal of medium, the cells were γ irradiated with a dose of 16 krad, and subsequently incubated in fresh medium at 37°C . After incubation times of 0 ($\bullet$), 20 ($\times$) or 40 ($\circ$) min the cells were scraped

off the dishes and lysed in 2% sodium dodecyl sulphate–0.02 M EDTA on top of alkaline sucrose gradients (5–20% sucrose, 0.1 M NaOH, 0.1 M NaCl), which were then centrifuged at 38,000 r.p.m. for 60 min in an SW 50.1 rotor (Beckman L2–65B centrifuge). Fractions were collected and radioactivity measured as described previously¹⁴. Sedimentation is from right to left. c, Normal (1BR, $\times$) and ataxia (AT4BI, $\bullet$) cells were labelled either as in (a) and (b) or in some experiments with $0.2\text{ }\mu\text{Ci ml}^{-1}$ ^{14}C -thymidine (1BR) and $5\text{ }\mu\text{Ci ml}^{-1}$ ^3H -thymidine (AT4BI). The cells were γ irradiated with 27 krad and incubated for various times. They were then scraped off the dishes and lysed in 2% lithium dodecyl sulphate containing 1 M LiCl, 0.01 M sodium citrate, 0.02 M EDTA, pH 9–9.5, layered over 5–20% sucrose gradients containing 1 M LiCl, 0.01 M sodium citrate, pH 9–9.5. The gradients were underlaid with 0.5 ml 60% sucrose saturated with CsCl. In double-labelling experiments DNA from the cells labelled with different isotopes were centrifuged in the same gradient. After centrifugation at 20,000 r.p.m. for 1 h fractions were collected and the radioactivity was measured. Two peaks were obtained, one which sedimented rapidly (RSP) and was held on the sucrose/CsCl shelf, the other being a slower sedimenting free DNA peak. The proportion of the total radioactivity sedimenting in the RSP is plotted against the time of post-irradiation incubation at 37°C . Results shown are the mean of four experiments. Variation between experiments was much greater than the difference between the two cell lines in any one experiment.

of the rejoining of double-strand breaks. On this basis, we can infer that human fibroblasts, like Chinese hamster cells¹¹, can rejoin double-strand breaks. Preliminary findings of our experiments (which will be reported in detail elsewhere) indicate that the rate of reformation of the RSP (rejoining of double-stranded breaks) is similar in normal and AT cell strains (Fig. 2c).

The human ultraviolet-light sensitive disorder xeroderma pigmentosum has been of great value in elucidating the relationship between ultraviolet sensitivity, defective repair of ultraviolet-induced damage to DNA, enhanced frequency of malignancy and increased levels of cellular mutagenicity¹². The AT syndrome provides an instance where clinical manifestations of damage by ionising radiation³⁻⁵ is reflected at the cellular level. Our preliminary studies on rejoining of DNA strand breaks have not revealed any deficiency in these processes in AT cells. Involvement of DNA damage in the AT syndrome is, however, suggested by the fact that AT cells are also more sensitive than normal cells to actinomycin D, mitomycin C and methyl methanesulphonate (personal communication from D. I. Hoar and P. Sargent), and to the chromosome-breaking effects of ionising radiation^{6,7}. It is hoped that studies with AT cells will promote a better understanding of the molecular basis of the response of cells to ionising radiation and other mutagens.

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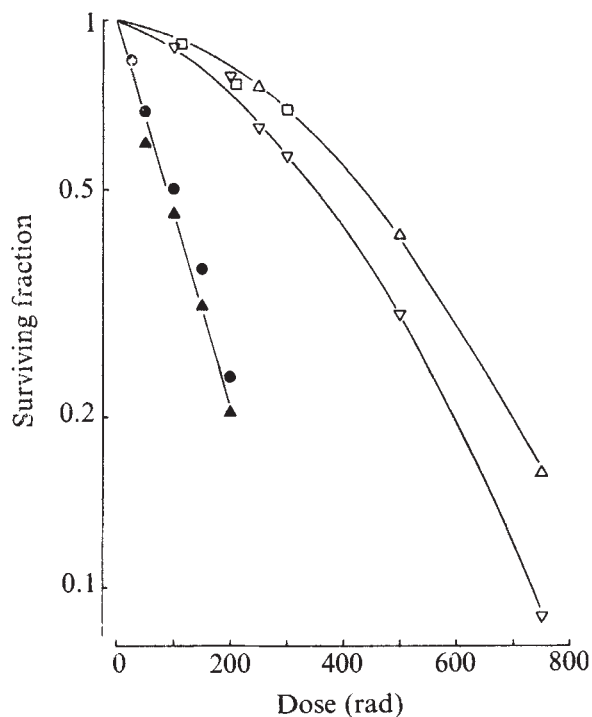


Fig. 1 Survival curves of irradiated human diploid and V79 Chinese hamster cells. Closed symbols, human cells; open symbols, hamster cells. Radiations: Δ ∇ $\blacktriangle$ ^{60}Co γ rays; $\bullet$ 250 keV X rays; $\square$ 5 keV μm^{-1} protons. HF19 human diploid fibroblasts were initiated from female embryonic lung tissue and maintained in monolayer culture⁹. The stock of V79 Chinese hamster cells was originally obtained from Dr E. H. Y. Chu; it was held at liquid nitrogen temperature and cells were removed and grown in monolayer culture for several days before an experiment. Both cell types were grown in Eagles MEM plus 10% foetal calf serum (Gibco-Biocult) and exponentially-growing populations were irradiated on plastic Petri dishes at the following dose rates: γ rays, 120 rad min^{-1} ; X rays, 50-250 rad min^{-1} ; protons, 70-160 rad min^{-1} . Hamster cells were also irradiated as suspensions in growth medium, made up immediately after trypsinising monolayers of cells (∇). After irradiation cells were incubated on plastic Petri dishes for 7 d (hamster cells) or 14 d (human cells) in a gas phase of 5% CO_2 in air at 37 °C. Clones arising on the dishes were stained and counted using a low power microscope. The curves shown are representative of data obtained in several experiments.

been reported, even by workers using the same cell line and method of mutant estimation^{1,2}. Also there has been no unequivocal demonstration of radiation-induced mutation in freshly isolated diploid (homonuclear) cell cultures^{3,4}. We consider that these discrepancies arise primarily from methodological difficulties, and report here the similarity of mutation frequency induced by ionising radiation in an established (heteronuclear) cell line used in many previous studies (Chinese hamster V79) and in human diploid cells (early passage foetal lung cells). The mutational response of the two cell types can be considered as identical when the differences in sensitivity to the inactivating effects of radiation are taken into account.

Mutant clones having reduced activity of the purine salvage enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT; EC 2.4.2.8) were selected by their resistance to the cytotoxic purine analogue 6-thioguanine. Quantitative estimation of induced mutation to purine analogue resistance is influenced by such factors as the efficiency of the chosen analogue, the concentration of that analogue, cell plating density, and time of addition of the analogue to the cells after mutagenic treatment ('expression time'⁵). In the present experiments the optimum conditions for each of the cell types were established by varying each factor in turn until a maximum mutant frequency was found, and by reconstruction experiments⁶ involving artificial mixtures of isolated mutants and wild-type cells. The

Mutation induction and inactivation in mammalian cells exposed to ionising radiation

MAMMALIAN cell cultures are used to estimate mutation induction by radiation and chemical agents in the belief that they will provide relevant data for assessing risk to man and insights into the mechanisms of mutagenesis in eukaryotic cells. It might be anticipated that these estimates would be obtained with the precision characteristic of studies with microorganisms, but very large variations in the response of mammalian cells to ionising radiation have

results of these experiments will be published in full elsewhere. During the expression time, microcolonies are formed from the cells surviving treatment and in many previous experiments the analogue was added directly to these and not to single cells. This method does not always allow the recovery of all the induced mutants, and in the present experiments cells were maintained in exponential growth during the expression time and then replated into analogue-containing medium. The viability of the cell population was also estimated on replating, and the mutant frequencies are expressed per viable cell at this time.

Tests on several isolated human cell mutants showed that all had less than 2% of wild-type HGPRT activity (assessed by uptake of ^{14}C -hypoxanthine into whole cells⁶), and were sensitive to the glutamine analogue azaserine⁷. Only some of the hamster cell mutants, however, showed this extreme phenotype. Others had approximately 15% of wild-type HGPRT activity and could be distinguished by their ability to survive azaserine treatment. These were found not to increase in frequency with radiation dose and are excluded from the data given in Figs 2 and 3.

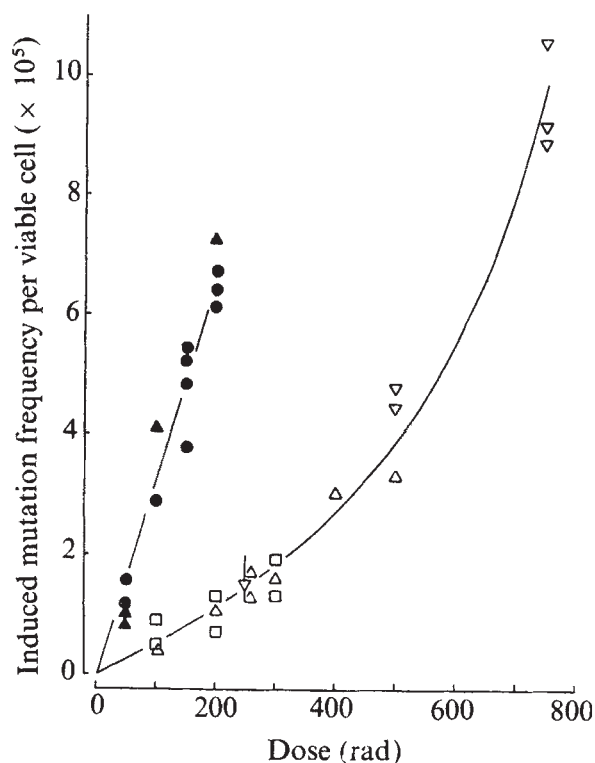


Fig. 2 Induction of HGPRT-deficient mutants of human diploid and V79 Chinese hamster cells by radiation. Symbols, growth conditions and irradiation procedures are as in Fig. 1. After irradiation, expression time growth was in normal medium for 2–3 d (hamster cells) or 7–11 d (human cells). Cells were then transferred to 9-cm plastic Petri dishes or bulk cell culture vessels (Sterilin) containing normal medium and 6-thioguanine (Sigma) at $0.7 \mu\text{g ml}^{-1}$ (hamster cells) or $2 \mu\text{g ml}^{-1}$ (human cells). Cell densities in selective medium were $2 \times 10^3 \text{ cells cm}^{-2}$ (hamster cells) or $10^3 \text{ cells cm}^{-2}$ (human cells). Cells were incubated in selective medium for 12 d (hamster cells) or 21 d (human cells). Human thioguanine-resistant clones were stained and counted using a low power microscope, and hamster thioguanine-resistant clones were subjected to a second selection in azaserine medium (azaserine $2 \times 10^{-5} \text{ M}$, hypoxanthine $5 \times 10^{-5} \text{ M}$, thymidine $5 \times 10^{-6} \text{ M}$ in normal growth medium). HGPRT-negative hamster mutant clones were found to degenerate and detach from the plastic surface within 7 d of adding this medium, and were counted using dark ground illumination with a low power microscope. For a given dose, each data point shown is from a separate experiment. The symbol and vertical bar at 250 rad indicate the mean and range of 9 separate experimental determinations for hamster cells.

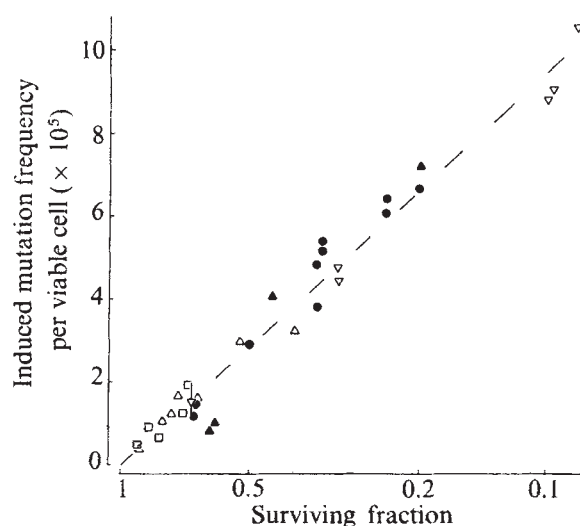


Fig. 3 The relationship between surviving fraction and induced mutation frequency in irradiated human diploid and V79 Chinese hamster cells. Symbols are as given in Fig. 1. The data points of Fig. 2 have been plotted against the log of the surviving fractions obtained in the same experiments.

Figure 1 shows the survival curves of the two cell types after ionising radiation treatment; they are clearly different, with the human cells showing an unshouldered exponential curve ($D_0 \sim 120 \text{ rad}$, $n=1$) and the hamster cells showing a shouldered response ($D_0 \sim 220\text{--}260 \text{ rad}$, $n \sim 3$). These curves are similar to those previously published for radiation of low linear energy transfer^{1,2,4}. The shapes of the curves of induced mutation frequency against dose are also different for the two cell types (Fig. 2), the human cells exhibiting a linear induction ($4 \times 10^{-7} \text{ mutants rad}^{-1}$) and the hamster cells a cumulative response (5×10^{-8} to $3 \times 10^{-7} \text{ mutants rad}^{-1}$). The absolute number of mutant colonies recovered in these experiments was increased for both cell types by up to a factor of six over the dose range studied, and the pre-existing mutants were found to have no selective advantage in the conditions used.

A plot of induced mutation frequency against the logarithm of the surviving fraction for human cells must show a linear relationship, since these quantities are both linearly related to dose. A corresponding relationship was not anticipated for hamster cells, but Fig. 3 shows that it was found and, moreover, that the linear relationships shown by the two cell types were indistinguishable.

The relationships of Fig. 3 indicate that the probabilities of conversion of radiation-induced lesions to inactivation or to mutation remain in a fixed ratio, in spite of differences in the radiation response of the cell species. The full implications of this finding, however, remain obscure because of our present ignorance of the molecular processes occurring in these cells after irradiation. It is possible that the survival-mutation relationship will be altered in radio-sensitive mammalian cell variants, such as the cultures derived from human patients with Ataxia telangiectasia¹⁰, and a study of the properties of these variants may yield information on the mechanism of conversion of radiation-induced lesions.

The observed relationship between survival and the frequency of mutation to thioguanine resistance may be used to predict the frequency against dose curve for similar mutations in other cell lines from a knowledge of their survival curves alone. An extension of this approach to other mutations may be useful in predicting the genetic risk to man from exposure to ionising radiation.

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Structural alterations in fish epidermal mucus produced by water-borne lead and mercury

The epidermal mucus of fish regulates swimming speed by controlling the hydrodynamic resistance of the skin surface^{1,2}. The mucus is also presumed to serve as a defence against pathogenic organisms and is intimately associated with osmoregulation^{3,4}. Because water-borne lead and mercury accumulate in the epidermal mucus of fish^{5,6}, it is important to ascertain whether this phenomenon produces deleterious alterations in the mucus. No detailed information is, however, available to evaluate whether such alterations occur on exposure of fish to lead and mercury or whether a capability exists to depurate these metals.

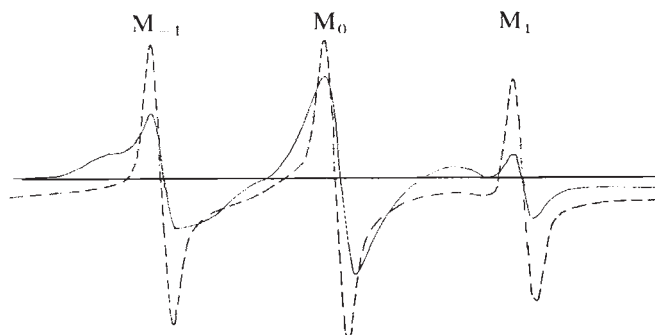


Fig. 1 Representative example of the change in electron spin resonance (ESR) spectra of fish mucus on exposure to low levels of heavy metals. ESR spectra of the control (—) and mercury-exposed (---) mucus (1 p.p.m. HgCl_2) labelled with N,N-dimethyl-N-dodecyl-N-tempylammonium bromide. Spectra were obtained at 20 °C using a Varian E-3 spectrometer. The labelled samples were placed in Kimax 51 capillaries (1.6-1.8 mm diameter). The rotational correlational times were calculated using the equation:

$$\tau = \left[\left(\sqrt{\frac{h_0}{h_1}} + \sqrt{\frac{h_0}{h_{-1}}} \right) - 2 \right] \left[\frac{4\pi\sqrt{3}W_0}{b^2} \right]$$

where $b = 3.06 \times 10^8 \text{ s}^{-1}$; h_1 , h_0 and h_{-1} are the height of peaks M_1 , M_0 and M_{-1} respectively; W_0 is the width of the centre hyperfine line M_0 in Hz.

We report that small concentrations of lead and mercury in the surrounding water produce significant alterations in the properties of epidermal mucus of rainbow trout (*Salmo gairdneri*) as revealed by electron spin resonance (ESR) spectrometry. Large differences were shown to exist in the degrees of accumulation and depuration of mercury and lead, but the observed structural alterations in the mucus still remain even after a period of depuration.

The rainbow trout ($350 \pm 25 \text{ g}$) were maintained in flow-through conditions at $10 \pm 0.5 \text{ °C}$. Experimental and control groups, each consisting of ten fish, were used. The experimental group was exposed to concentrations of HgCl_2 and PbCl_2 varying from 0.1 to 1.0 p.p.m. ($\mu\text{g g}^{-1}$). In this concentration range, the pH values (5.8 ± 0.05) of the surrounding water did not change significantly. Constant levels of metal chloride solutions

were maintained by continually mixing appropriate amounts of stock solutions with fresh water using a proportionating pump (Technicon Instrument Corp., New York) at a flow rate of 2 l min^{-1} . In these initial experiments, the exposure period was chosen as the time for 20% of the fish in the experiment to die. The exposures varied from 41 to 88 h for PbCl_2 and 14 to 65 h for HgCl_2 . Fish anaesthetised with dilute solutions ($75 \mu\text{g g}^{-1}$) of ethyl *m*-aminobenzoate methane sulphonate (MS-222; Crescent Research Chemicals, Inc., Scottsdale, Arizona) were suspended vertically from the mandible, and excess water was drained. The epidermal mucus ($7.3 \text{ mg per g of fish}$) was then removed by gently scraping the skin surface with a spatula. The protein content of each sample of mucus was measured⁸ and the values obtained ($9.3 \pm 0.9 \text{ mg protein g}^{-1} \text{ mucus}$) were fairly constant, indicating that the mucus was obtained under reproducible conditions. Metal concentrations were determined by atomic absorption spectrophotometry^{8,9}. Alterations in the properties of the mucus were evaluated by ESR using a free radical probe (N,N-dimethyl-N-dodecyl-N-tempylammonium bromide) for polar sites. The mucus was stirred with the probe, in a ratio of 150 : 1 w/w for 12 h at 5 °C. Unassociated probe was removed by dialysis (4,000-6,000 molecular weight unit pore size) against distilled water for 12 h. By measuring the metal content of a sample before and after dialysis it was determined that this did not cause significant losses of bound lead or mercury. Rotational correlation times (τ) were calculated¹⁰ for each ESR spectrum of mucus (Fig. 1) obtained from both control and exposed fish. The correlation time is inversely related to the tumbling frequency of the probe. A rotational correlation time of 10^{-11} s indicates rapid tumbling of the probe in a rather 'fluid' environment. A value of 10^{-8} s ,

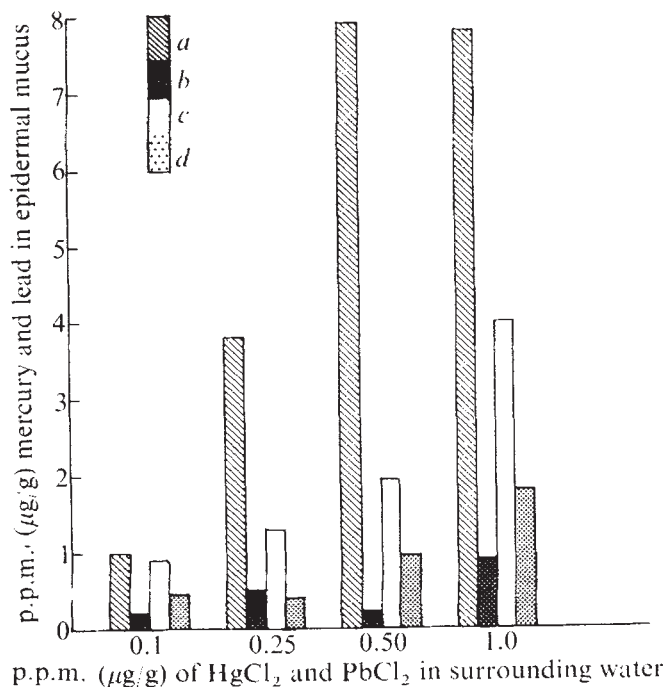


Fig. 2 Levels of mercury and lead in the epidermal mucus of rainbow trout exposed to HgCl_2 and PbCl_2 solutions^{9,10}. a, Accumulation of mercury in mucus after exposure to HgCl_2 ; b, concentration of mercury remaining in mucus after 24 h of depuration; c, accumulation of lead in mucus after exposure to PbCl_2 ; d, concentration of lead remaining in mucus after 24 h of depuration.

however, indicates a highly immobilised probe in a relatively 'rigid' environment¹⁰. The rotational correlation time of $2 \times 10^{-9} \text{ s}$ obtained for mucus from the control fish indicates a partially immobilised probe.

Exposure of fish to the aqueous PbCl_2 solutions showed that the mucus accumulated 0.9-4.0 p.p.m. of lead. Comparable experiments with HgCl_2 resulted in 1.0-7.9 p.p.m. of mercury

(Fig. 2). When the fish exposed to lead were placed in metal-free water for 24 h, the mucus depurated a maximum of 70% of the sequestered metal. In the mercury-exposed fish the maximum value obtained for the mucus depuration was 97% (Fig. 2). At each level of exposure the fish were able to depurate from the mucus a much higher percentage of mercury than lead. Accordingly, it seems that lead is more persistent in the epidermal mucus than mercury. No obvious trend exists in the degree of depuration relative to the level of exposure.

The preferential depuration of mercury seems to be related primarily to the fact that HgCl_2 promotes the formation of copious amounts of mucus, as shown with goldfish⁶. Our visual observations confirm this finding. The possibility that the preferential depuration results from the lead being the more tightly bound to the mucus was considered. Such an occurrence seems unlikely because our dialysis experiments with metal-exposed mucus did not result in significant losses of either mercury or lead. It seems therefore, that the decrease in the levels of lead and mercury in mucus after 24 h in metal-free waters represents a sloughing-off of the metal-complexed mucus, with subsequent replacement by metal-free mucus.

The ESR spectra of mucus obtained from the fish exposed to lead and mercury revealed that substantial alterations in rotational correlation times occurred relative to values obtained for mucus from control fish (Fig. 3). These differences were evident at each exposure level. Dramatic decreases in the τ values for concentrations between 0 and 0.1 p.p.m. of HgCl_2 were obtained, suggesting that the mobility of the spin label had substantially increased. Exposure of 0.1–1.0 p.p.m. of HgCl_2 resulted in marked increase of the τ values over the minimum value, obtained at 0.1 p.p.m. Thus, a tendency exists to reverse the trend toward the mobility of the probe at concentrations greater than 0.1 p.p.m. Nevertheless, the overall effect of the mercury was to produce a substantial

increase in the fluidity of the environments around the nitroxide probe, as evidenced by much lower values obtained for the exposed mucus than for the control (Fig. 3). In essence, severe changes were induced in the structure of polar sites in the mucus by the small concentrations of inorganic mercury in the water. The ESR data for the fish exposed to lead reveals that the fluidising process was equally dramatic; however, no obvious trend in the magnitude of τ values was observed (Fig. 3). When epidermal mucus from control fish was dialysed against HgCl_2 and PbCl_2 solutions (up to 25 p.p.m.), τ values obtained were much lower than those for normal mucus¹¹. Accordingly, the fluidisation observed in the metal-exposed mucus seems to be due to a direct interaction between the mucus and the metals.

The question arose whether the fluidisation of mucus, as shown by ESR, persists after depuration of the metals. The data given in Fig. 3 show that the τ values are still substantially lower than the control value even after 24 h of depuration. Moreover, with the exception of the 1.0 p.p.m. PbCl_2 exposure, the τ values were consistently lower for the depurated mucus than for the exposed mucus before depuration. Accordingly, the metal-induced damage of the mucus structure persists even after the metals are removed from the surrounding water. Further studies are required to determine whether the observed effects on the epidermal mucus are reversible over longer periods of time.

The epidermal mucus of fish contains glycoproteins as important structural units^{3,12}. Thus, it seems that the observed changes may involve interactions of the metals with polar sites in these molecules. The possibility exists that the fluidisation of the mucus may alter the delicate rheological properties¹ of the epidermal mucus. Such an occurrence may result in changes in the hydrodynamic resistance and other vital processes of this medium.

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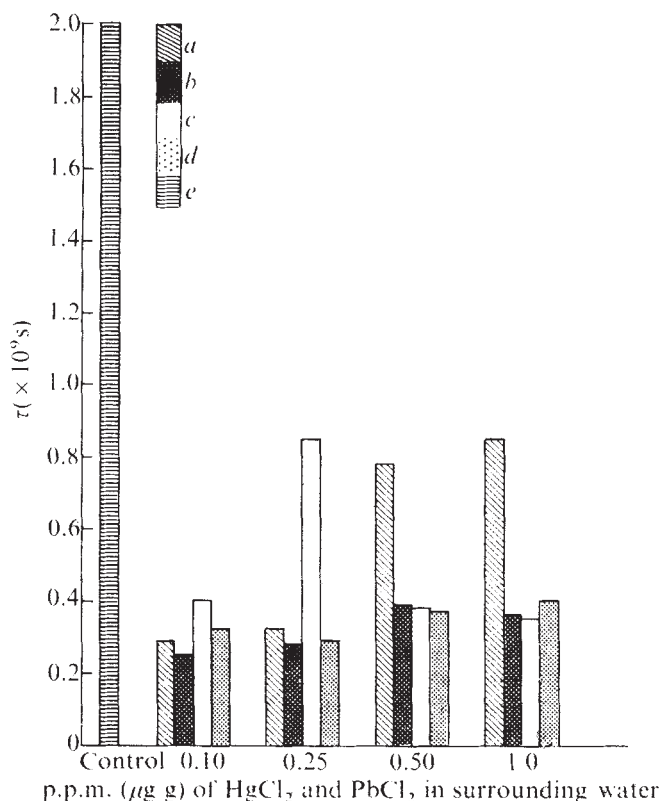
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Fig. 3 Rotational correlation times (τ values) obtained from ESR spectra of epidermal mucus from control fish and metal-exposed fish. a, τ values for mucus of fish exposed to HgCl_2 ; b, τ values for mucus of mercury-exposed fish after depuration of 24 h; c, τ values for mucus of fish exposed to PbCl_2 ; d, τ values for mucus of lead-exposed fish after depuration of 24 h; e, τ value for mucus of control fish was 2.0×10^{-9} s.



Persistence of CMV genome in lymphoid cells after congenital infection

THE persistence in a latent state of the herpes simplex virus in neuronal cells¹⁻³ and of the Epstein-Barr virus (EBV) in lymphoblastoid cells^{4,5} has been documented. Cytomegaloviruses (CMVs) have been isolated in the past from leukocytes of congenitally infected babies⁶ and found in peripheral leukocytes by *in situ* RNA-DNA cytohybridisation (C. H. Huang, P. Neiman and J. S. Pagano, unpublished data). EBV-positive lymphoblastoid cell lines have been established from three of 12 patients with CMV mononucleosis^{7,8} but the possible persistence of CMVs in these lines was not documented. We have now found an

unexpressed CMV genome in an EBV-positive lymphoblastoid cell line.

This line was established from a 9-month-old male infant (S.D.), born with a petechial rash and periventricular calcifications from a primiparous apparently healthy mother. Cytomegaloviruses were isolated from four successive urines of this baby. Three of these urines were collected from birth to age 5 months, the fourth was collected at age 19 months. Peripheral leukocytes collected successively at age 4, 9, 15 and 21 months were all established as permanent lymphoblastoid cell lines. These had B-cell characteristics, were of male karyotype and were diploid with 46 chromosomes. The percentage of polyploidy of 18%, however, was slightly greater than normal. All four lines are positive for EBV soluble (S) antigen by complement fixation and for the EBV nuclear (EBNA) antigen by anti-complement immunofluorescence but are negative so far, even after treatment with bromodeoxyuridine for EBV viral capsid antigen⁹. Cytomegalovirus antigens have not been detected using a CMV-positive EBV-negative human serum in both standard indirect and anti-complement immunofluorescence tests. Herpesvirus particles have not been seen by negative staining electron microscopy in concentrated extracts of the cells. Cell extracts and supernatants for the second line did not yield CMV on human embryo fibroblast cells known to support the growth of the AD-169 CMV and the CMV from the infant's urine. The first line (EB-SD-1) contained eight EBV genome equivalents per cell as detected by DNA-DNA reassociation kinetics analysis¹⁰, but was negative for CMV DNA by the same method¹¹ as well as by cRNA-DNA membrane hybridisation^{11,12} (Table 1). The second line (EBCM-SD-2) was positive not only for EBNA but also for EBV early antigens and contained 8–10 CMV genome equivalents (Table 1) and 9.5 EBV genome equivalents (Fig. 1) per cell. As Fig. 1 shows, however, the EBV genomes in both lines SD-1 and SD-2 only shared 65–70% homology with that of the HRIK virus.

As far as we know this is the first time that the CMV genome has been found unexpressed in a lymphoblastoid cell line. We cannot eliminate the possibility that a few non-infectious particles of CMV or CMV antigens in amounts undetectable by our immunofluorescence methods are present in these lines. To rule out the possibility that the virus in the urine could be EBV or an unusual transforming strain of CMV, we inoculated cord blood leukocytes with the virus: the cells were destroyed rapidly. The fact that EBV DNA from EB-SD-1 and EBCM-SD-2 lines shares only 65–70% of homology with EBV DNA from the HRIK line (Fig. 1) suggests either that the EBV genome in SD cells is defective or that there are different EBV strains. These possibilities are supported by data showing that EBV from HRIK and from B95-8 lines can be distinguished by different biological properties^{14,15} and unequal genome size¹⁶.

Table 1 CMV and EBV genome equivalents in the cell DNA of the two first lines from infant S.D.

	Cell line	
	First (EB-SD-1)	Second (EBCM-SD-2)
CMV genome equivalents per cell		
cRNA-DNA membrane hybridisation	<1	8*, 9.5†
DNA-DNA reassociation kinetics analysis	<0.4	
EBV genome equivalents per cell		
DNA-DNA reassociation kinetics analysis	8	9.5

*³²P-cRNA complementary to CMV strain AD-169 DNA.

†¹²⁵I-cRNA complementary to CMV strain AD-169 DNA.

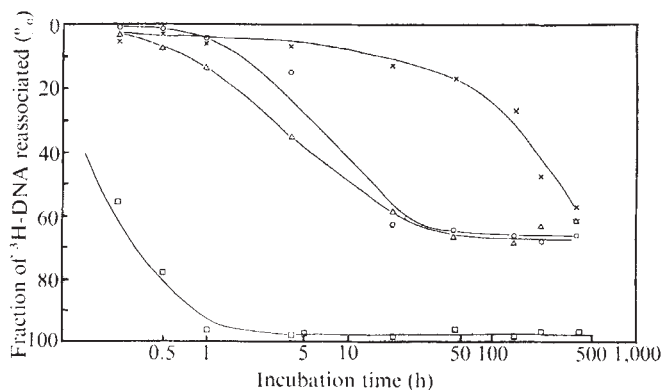


Fig. 1 DNA-DNA reassociation kinetics analysis of S.D. cell DNA and EBV ³H-DNA. Sonically disrupted ³H-labelled EBV DNA (purified from HRIK cells, 0.02 µg (3.4 × 10⁴ c.p.m.), and 2 mg of calf thymus DNA (×), EB-SD-1 cell DNA (○), EBCM-SD-2 cell DNA (△), or HRIK DNA (□) were mixed and denatured in the presence of 0.01 M Tris-HCl, pH 7.4 and 0.0025 M EDTA. The salt concentration was then adjusted to 1.2 N NaCl. Hybridisation was carried out at 66 °C, and the fraction of reassociated ³H-DNA was analysed by S1 enzyme differential digestion¹⁰.

Work is in progress to determine whether CMV played a part in the establishment of the second lymphoblastoid cell line and if it can be rescued from these cells by the co-cultivation and fusion method. Finally, labelled CMV DNA from the urine virus will be prepared and used in a reassociation kinetics assay in the presence of SD 1 and 2 cell DNA. There is no detectable (less than 5%) homology between the DNA of HRIK-EBV and AD-169 CMV¹⁰. Other strains of EBV and CMV, however, will have to be examined to see if they are related.

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Note added in proof: No infectious CMV could be recovered from SD2 cells following three successive cocultivation experiments with human embryo fibroblasts, but an EBNA positive lymphoblastoid cell line of female karyotype was obtained by cocultivating lethally X-irradiated SD2 male cells with cord blood lymphocytes from a female newborn. The latter experiment will be repeated using EBV positive CMV negative serum in an attempt to neutralise EBV and obtain a CMV transformed cord blood lymphocyte cell line.

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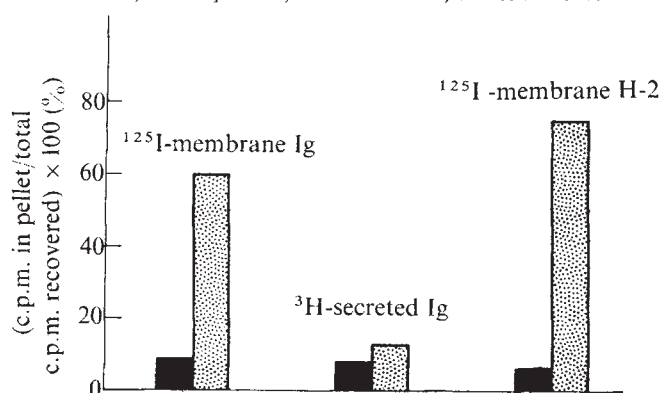
Are immunoglobulins integral membrane proteins?

IMMUNOGLOBULINS (Igs) are present on the surface of murine bone-marrow derived (B) lymphocytes¹⁻³. Combination of the membrane Ig and a specific antigen, together with the arrival of a signal from thymus-derived (T) lymphocytes induces B cells to divide and differentiate into antibody secreting plasma cells⁴. To understand the mechanism of that induction, it is necessary to know how Ig is attached to the cell surface.

Singer and Nicolson⁵ classified membrane proteins as integral or peripheral on the basis of their solubilities. Integral proteins, as defined by them, can only be extracted from membranes by detergents or organic solvents, and require the continued presence of these agents for solubility. Peripheral proteins can be extracted with moderate salt concentrations, slight alterations of pH, EDTA, and other mild reagents. Detergents are not required to maintain the solubility of peripheral proteins. Membrane Igs have been extracted with the non-ionic detergent NP-40 (refs 2 and 6) and with 8 M urea⁸ but not with potassium chloride, sodium sulphate, and sodium trichloroacetate⁶, and Ig remains associated with the purified plasma membrane of lymphoma cells despite extensive washing⁶⁻⁸. These findings suggest that membrane Ig may be an integral protein of the lymphocyte plasma membrane.

We sought to test whether membrane Ig was an integral protein by determining whether detergent was required for its solubility. Triton X-100 has been shown to be easily removed from protein solutions with Bio-beads SM-2 (BioRad)⁹. Since NP-40 is similar in structure to Triton X-100, we attempted to remove NP-40 from solutions with these beads. When 2.0 ml of 0.5% NP-40 in phosphate-buffered saline (PBS) were mixed with 0.6 g Bio-beads SM-2 (prepared according to Holloway's procedure⁹) and gently agitated at 4 °C for 120 min, the concentration of NP-40 in the solution declined to less than 0.001%, determined spectrophotometrically (at 277 nm). The effectiveness of detergent removal was also measured by testing the ability of solutions to haemolyse sheep erythrocytes. Using this assay, Bio-bead treatment resulted in detergent solutions of

Fig. 1 Distribution of membrane Ig, secreted Ig and membrane H-2 antigen in high speed pellet for detergent present and absent. Ig was identified by sandwich immunoprecipitation with rabbit anti mouse κ , using rabbit anti- α X as control¹¹. H-2 was identified in 'Ig-cleared' supernatants with ABY anti ASL-1 using C3H/BALB/c normal mouse serum as control¹⁶. Solid columns, NP-40 present; dotted columns, NP-40 removed.



about 0.005–0.010%, probably near the critical micelle concentration of the detergent. Similar results were obtained if NP-40 (2 ml 0.5%) had been used to lyse spleen cells (1×10^8).

Spleen cells from 6–12-week-old BALB/c mice were treated with Na¹²⁵I, lactoperoxidase and H₂O₂^{3,10}, and then washed. Iodinated cells were lysed with NP-40. After removal of nuclear material and debris by centrifugation (2,000g for 15 min), the supernatant was divided equally into two. Bio-beads were added to one portion and both aliquots were dialysed overnight against phosphate-buffered saline (PBS) at 4 °C. The samples were freed of beads and centrifuged at 100,000g for 90 min. The pellet of the bead-treated sample consisted of a translucent brown layer covered by an opaque white precipitate. Only the opaque precipitate was visible in the no-bead control. The pellets were treated with 0.5% NP-40-PBS for 15 min at 37 °C, and centrifuged at 2,000g for 15 min to remove insoluble debris. The high speed supernatant and the NP-40 resolubilised pellet were assayed for membrane Ig by immunoprecipitation^{11,12}. NP-40 was added back to the supernatant of the bead-treated sample to produce a final concentration of 0.5%, and provide similar conditions during immunoprecipitation.

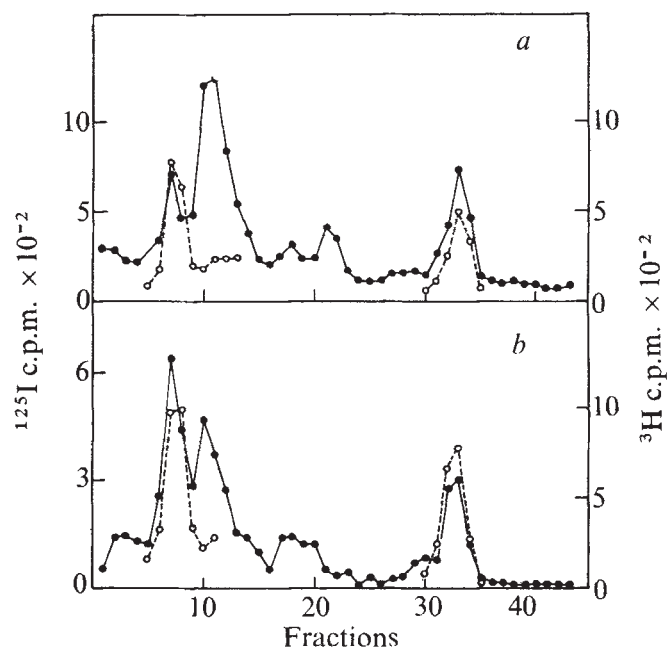


Fig. 2 SDS-polyacrylamide gel electrophoresis of membrane Ig (●) in high speed supernatants (a) and pellets (b) after detergent removal. Immunoprecipitates were dissolved in SDS, reduced with 2 mercaptoethanol, and electrophoresed in 12.5% acrylamide gels in the presence of SDS¹³. ³H-secreted Ig (○) was used as marker for μ and L chains.

The results of one representative experiment are shown in Fig. 1. Of the ¹²⁵I-Ig in the control, only 9% were in the high speed pellet. Some nonspecific trapping occurred since pellets were not washed before solubilisation. In contrast, after bead treatment, 60% of the recovered Ig was pelletable. In four experiments, the proportion sedimented varied from 40 to 60%. The recovery of ¹²⁵I-Ig in control and treated samples was always close to 100%. To control for direct effects of the beads on Ig solubility, we used Ig secreted by spleen cells. The secreted Ig had been labelled with ³H by incubation of cells with ³H-L-tyrosine^{10,13}. The dialysed cell secretions were made to 0.5% in NP-40 and the bead treatment was the same as before. In both control and treated samples only about 10% of the ³H-Ig was in the pellet fraction (Fig. 1). The remaining 90% were in the supernatant. To control for possible effects of polymerisation on the ability of pentameric IgM to aggregate, a secretion was partially reduced with 0.5 mM dithioerythritol¹⁴. After alkylation with 2.5 mM iodoacetamide and addition of NP-40 to 0.5%, one half of the sample was treated with beads as described above. The other half served as control.

The reduction yielded u_2L_2 and some higher polymers as determined by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis¹². In both samples, 75% of the immunoprecipitable radioactivity was not sedimentable. Thus, the presence of detergent was required for the solubility of membrane Ig, but not for the solubility of secreted Ig either in the monomeric or polymeric forms.

That not all of the membrane Ig could be pelleted in the above experiments may indicate that sedimentation did not completely separate aggregated molecules from monomers. This view is supported by the distribution of H-2 alloantigen in the high speed pellet and supernatant. This protein, which is thought to be an integral membrane protein¹⁵, was identified by immunoprecipitation from Ig cleared lysates¹⁶. Only 5% of the H-2 was pelletable in the presence of NP-40, but in its absence 75% became sedimentable (Fig. 1). That this value, like the value for membrane Ig, is not 100%, suggests that sedimentation may only separate larger sized aggregates. A preliminary analysis of a detergent-free high speed supernatant by gel filtration on Sepharose 4B showed that the membrane Ig remaining in this supernatant was present in aggregates of from 3×10^5 to 1×10^6 daltons (little or no membrane Ig radioactivity was in the 2×10^5 dalton range expected for monomeric IgM).

There are two classes of Ig on the surface of murine lymphocytes, IgM and an IgD-like molecule^{11,17}. The two classes can be distinguished by the mobility of their heavy (H) chains during electrophoresis in the presence of SDS in 7.5% and higher acrylamide concentration gels. The incomplete sedimentation of membrane Ig after detergent removal could have been due to the aggregation of only one class of membrane Ig. We therefore subjected the membrane Ig immunoprecipitated from high speed supernatants and NP-40 resolubilised pellets to SDS-polyacrylamide gel electrophoresis. 3H -secreted Ig was used as an internal marker for μ and light chains. As can be seen in Fig. 2, both μ and δ chains were present in the supernatant and pellet. The higher ratio of μ/δ in the pellet (and the reverse in the supernatant) compared with that of the untreated control was observed in all six experiments. The 3H - μ chain markers (from secreted IgM) in Fig. 2 moved slightly faster than the membrane μ chain whether the latter was from the supernatant or the pellet. The slight difference in mobility is highly reproducible, and is discussed in detail elsewhere¹².

We believe the most likely interpretation of the above results is that IgM and IgD are integral proteins which are structurally different from their secreted highly soluble counterparts. We cannot, however, exclude an alternative but less likely possibility that membrane Ig is a peripheral protein attached by non-covalent bonds to an integral protein in the membrane. Although our results suggest that IgM is attached directly to the lipid bilayer, only a small region of the molecule, perhaps a C terminal section of the μ chains, is probably involved in this interaction, since most of the molecule is accessible to lactoperoxidase² and antisera¹⁸ *in situ* on the surface of the cell.

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Circadian cycles in binding of 3H -alprenolol to β -adrenergic receptor sites in rat pineal

A β -ADRENERGIC receptor coupled to adenylate cyclase regulates the activity of rat pineal N-acetyltransferase, an enzyme involved in the biosynthesis of melatonin¹. At the onset of darkness there is an increase in the release of the neurotransmitter noradrenaline from sympathetic nerves^{2,3} which produces 30- to 50-fold increases in N-acetyltransferase activity⁴. During periods of exposure to light, nerve activity and noradrenaline turnover are lower than during periods of darkness^{2,3}, and N-acetyltransferase activity remains at its lowest level⁴.

Chronic reduction of the adrenergic input to the pineal gland, by denervation or by exposure of the animals to constant light, causes supersensitivity of N-acetyltransferase induction⁵, adenylate cyclase activation⁶, and cyclic AMP accumulation^{6,7} to stimulation by catecholamines. Pretreatment of the animals with isoproterenol or noradrenaline prevents the development of, or reverses, this supersensitivity, causing a relative subsensitivity^{8,9}.

Moreover, there are daily cycles of sensitivity in the induction of N-acetyltransferase by isoproterenol^{7,10} which are correlated with circadian cycles of noradrenaline turnover in the pineal gland³. The increase in sensitivity seen within a few hours of exposure to light is related to decreased sympathetic nerve activity, whereas the obverse is true after 12 h of exposure to darkness.

Recent work has established that 3H -(-)-alprenolol (a competitive β -adrenergic antagonist) binds with high affinity and stereospecificity to pineal¹¹ and other tissue membranes¹². This binding occurs at sites with characteristics indistinguishable from the β -adrenergic receptors which are coupled to adenylate cyclase. Here we report that the number of 3H -(-)-alprenolol binding sites in the pineal gland varies with a circadian periodicity inversely related to the cycle of neurotransmitter release. Furthermore, these cycles in the number of specific β -adrenergic binding sites correlate with, and probably contribute to, the circadian rhythm in the sensitivity of cyclic AMP accumulation to stimulation by catecholamines.

Male Sprague-Dawley rats (150-175 g, Zivic Miller) were kept in a light/dark regime (lights on from 0600 to 1800) for at least 5 d. 3H -(-)-alprenolol binding assay was performed at concentrations which saturated the specific binding sites, by a modification¹¹ of a method described for frog erythrocyte membranes¹². Cyclic AMP levels were measured¹³ 10 min after injection of saline or 1-isoproterenol-*d*-bitartrate (5 mg kg⁻¹, subcutaneously). The post-decapitation rise in cyclic AMP levels¹⁴ was avoided by homogenising the pineal glands within 30 s after killing the animals.

The number of specific 3H -(-)-alprenolol binding sites was measured at various times over a 24-h period, and shown to vary with diurnal periodicity (Fig. 1). The number of binding sites was lowest after 12 h in the dark. With the decrease in nerve activity that occurs at the onset of light, the number of binding sites increased. The injection of cycloheximide, an inhibitor of peptide synthesis, did not prevent the increase seen with light. Thus, the daytime reduction in β -adrenergic stimulation allows for an increase in the number of available receptor

sites by a process that does not require new peptide synthesis. With the onset of darkness, the process was reversed, and the number of binding sites rapidly decreased.

The elevation of cyclic AMP levels from baseline after an injection of isoproterenol was also examined over a 24-h period. The level of cyclic AMP in pineal glands from control animals did not vary significantly during a 24-h period (Fig. 2); the onset of light or darkness had no effect on the basal level of cyclic AMP. But the increase in cyclic AMP levels caused by the injection of isoproterenol varied markedly as a function of the previous lighting conditions. As the period of exposure to light increased, so did the accumulation of cyclic AMP after isoproterenol. With the onset of darkness, there was a decrease in the cyclic AMP response to isoproterenol measured at 2400 or 0600.

The variability of cyclic AMP accumulation after isoproterenol may be accounted for by variations either in the rate of production or destruction of cyclic AMP. Attempts to demonstrate diurnal variability in pineal phosphodiesterase activity have been unsuccessful. Small circadian variations in adenylate cyclase activation *in vitro* by catecholamines have been described previously¹⁴ and confirmed by us. Our data show that the number of specific β -adrenergic receptor sites varies in parallel with the accumulation of cyclic AMP in response to isoproterenol. Therefore, one mechanism contributing to these diurnal cycles in the accumulation of cyclic AMP is a change in the number of β -adrenergic receptor sites, which are coupled to adenylate cyclase. These changes apparently occur as a consequence of the cycle in neurotransmitter release.

We have shown that, in the pineal gland, pretreatment with isoproterenol rapidly reduces the number of β -adrenergic receptor sites and decreases the sensitivity of adenylate cyclase to β -adrenergic stimulation¹⁵. These changes are in agreement with the rapid desensitisation of adenylate cyclase by catecholamines described in other systems^{16,17}. In frog erythrocyte membranes such changes have also been correlated with the number of ^3H -(-)-alprenolol binding sites¹⁷. These rapid changes in the number of available β -adrenergic receptors do not require new peptide synthesis¹⁵.

The decrease in binding of ^3H -(-)-alprenolol following pharmacological stimulation is not due to changes in the apparent affinities of the receptor or adenylate cyclase for agonist or antagonists^{15,17}. The daily physiological variation in receptor number also is not accompanied by changes in receptor

Fig. 1 Diurnal variation in the number of specific β -adrenergic binding sites in rat pineal gland. Each time point is the average of multiple determinations made with each of two or three homogenates containing 7–10 pooled pineals. * $P < 0.05$; ** $P < 0.01$ compared with immediately preceding time point.

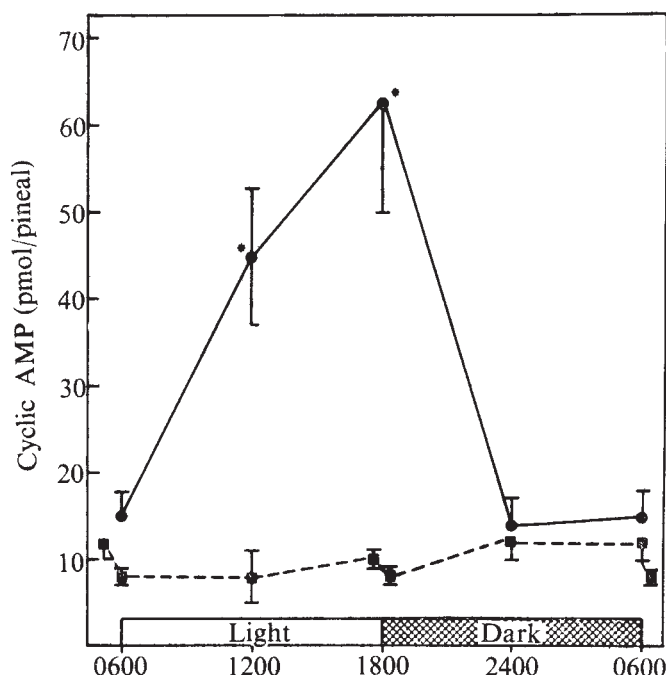
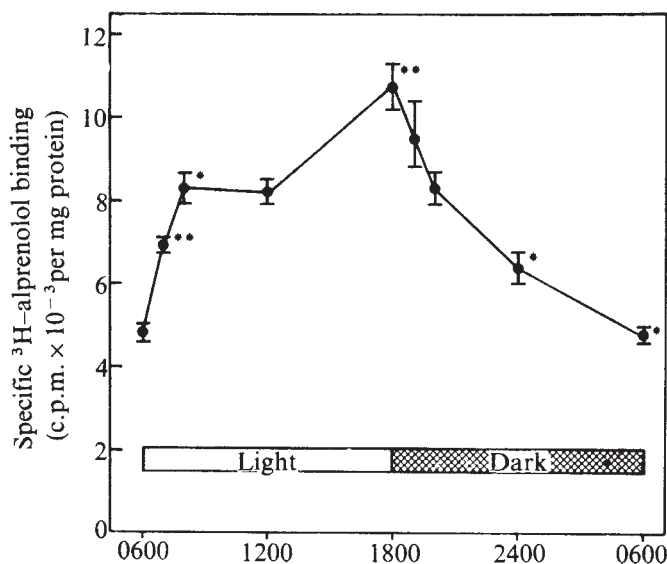


Fig. 2 Diurnal variation in the accumulation of cyclic AMP after isoproterenol administration in the rat pineal gland. At the various times indicated, rats were injected with saline (■) or 1-isoproterenol-bitartrate (●) (5 mg kg⁻¹), and pineal cyclic AMP levels measured ten minutes later. Results are means $\pm$ s.e.m. * $P < 0.05$ compared with controls by Student's *t* test.

binding affinity¹⁵. The variation in the number of binding sites is reflected as changes in the maximum stimulation of cyclic AMP attainable, thus potentially affecting the maximum induction of N-acetyltransferase.

The variations in the number of β -adrenergic binding sites and in the sensitivity of the pineal gland to catecholamines are reversible and result from physiological processes. The pineal gland could thus serve as a model for studying changes in drug sensitivity as a consequence of endogenous processes¹⁸. Furthermore, since the changes can also be produced by pharmacological manipulations, investigations in the pineal may have bearing on the phenomena of tachyphylaxis and drug tolerance.

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Direct identification and characterisation of β -adrenergic receptors in rat brain

MODULATION of cellular function by the autonomic nervous system is accomplished, at least in part, by the stimulation of β -adrenergic receptors by catecholamines at neuroeffector junctions¹. Activation of these receptors results in stimulation of adenylate cyclase with increased intracellular levels of cyclic AMP. The β -adrenergic receptors of a variety of non-neural tissues have been characterised indirectly by measurement of altered adenylate cyclase activity or of intracellular cyclic AMP in response to β -adrenergic agonists and antagonists. Direct characterisation of the β -adrenergic receptors in avian^{2,3} and amphibian⁴ erythrocytes and in canine heart⁵ has been accomplished by binding assays using radioactive β -adrenergic antagonists.

Catecholamines are thought also to be neurotransmitters mediating information transfer across synapses in the central nervous system (CNS)⁶. In the case of dopaminergic systems this mediation is thought to result from the stimulation of a dopamine receptor-coupled adenylate cyclase resulting in increased levels of cyclic AMP⁷. This dopamine-sensitive adenylate cyclase system has been extensively characterised in terms of structure-activity relationships and interactions with psychotropic drugs⁸. In contrast, although β -adrenergic receptor mediated changes in cyclic AMP have been observed in brain slices⁹ and electrophysiological evidence suggests that these receptors are important in mediating function in certain neurones¹⁰⁻¹², relatively little information is available concerning β -adrenergic receptor systems in the brain.

The β -adrenergic receptor of the CNS has not been fully characterised even by indirect methods because of the relative difficulty of measuring physiological responses and because, in contrast to most other tissues, the β -adrenergic receptor coupled adenylate cyclase in brain generally retains little or no hormone sensitivity after homogenisation¹³. Because of success in using ^3H -(-)-alprenolol, a β -adrenergic antagonist, to identify β -adrenergic receptors in canine myocardium and in frog erythrocytes, we used this compound to identify and to characterise the β -adrenergic receptor of the rat CNS.

Male Sprague-Dawley rats (225–300 g) were killed by decapitation. The brain was quickly removed, dissected and frozen at -70°C until used. Brain sections were homogenised in 5–8 volumes of cold buffer (0.25 M sucrose, 5 mM Tris-HCl, pH 7.4 and 1 mM MgCl_2) using 5–6 passes with a tight fitting Dounce homogeniser. The homogenate was centrifuged at 800g for 20 min at 4°C and the pellet was discarded. The supernatant was centrifuged at 4°C for 14 min at 14,000g and the resulting pellet was washed three times in the sucrose buffer. The pellet was resuspended in cold buffer (75 mM Tris-HCl, 25 mM MgCl_2 , pH 7.4) to a final protein concentration of 200–300 μg per 100 μl . Incubation of the crude membrane fraction, 18 nM ^3H -(-)-alprenolol (specific activity 17 Ci mmol⁻¹), and various agonists and antagonists were performed in a volume of 150 μl for 10 min at 37°C as described previously⁵. The binding reaction was terminated by rapidly adding 3 ml of ice cold buffer (50 mM Tris-HCl, 17 mM MgCl_2 , pH 7.4) and filtering through a Whatman GFC glass fibre filter mounted in a suction apparatus. The incubation tube was washed with additional 3 and 5-ml portions of cold buffer. The filter was subsequently dried and the trapped particles with their bound radioactivity were counted in a liquid scintillation counter using a Triton X-100 on toluene based scintillation fluid. The filter blank was less than 1% of added counts. "Specific" binding (counts displaced by 1×10^{-6} or 1×10^{-5} M (-)-propranolol) averaged approximately 70% of total bound counts. " ^3H -(-)-alprenolol binding" refers to specific binding.

Figure 1 shows the kinetics of ^3H -(-)-alprenolol binding to the cerebral cortex membrane fraction. Binding is approximately

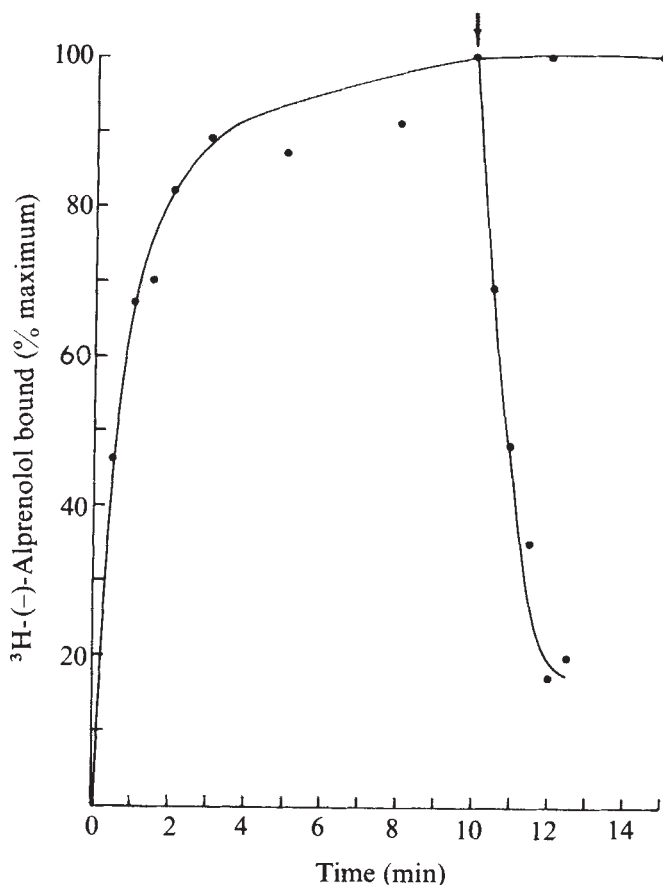
50% complete at the earliest time measured (30 s) and is virtually at equilibrium by 3 min. The reverse rate is also very rapid with a $t_{1/2}$ of approximately 30–60 s at 37°C .

Figure 2 shows that there are a finite number of specific binding sites in the rat cerebral cortex membrane fraction, as shown by the saturability of ^3H -(-)-alprenolol binding. Half maximal saturation occurred at 6.7 nM which provides an estimate of the dissociation constant (K_D) of (-)-alprenolol for the binding sites.

The specificity of ^3H -(-)-alprenolol binding is demonstrated in Fig. 3a. Thus (-)-propranolol is highly potent in competing for the binding sites. The order of potency of the β -adrenergic agonists in competing for the binding sites is (-)-isoproterenol > (-)-adrenaline = (-)-noradrenaline. The (+)-isomers of propranolol and the β -adrenergic agonists are approximately two orders of magnitude less potent in competing for the binding sites than are the (-)-isomers as shown in Fig. 3b.

In Table 1 the inhibition of ^3H -(-)-alprenolol binding by all drugs tested is summarised. Where possible, the concentration required to inhibit binding 50% (EC_{50}) was determined. This value is directly related (but not equivalent to) the K_D of the drug for the receptor. The receptor concentration used in the present assays was approximately 0.4 nM whereas the ^3H -(-)-alprenolol concentration is several times greater than K_D . Based on calculations derived from the methods of Cheng and

Fig. 1 Kinetics of ^3H -(-)-alprenolol binding to rat cerebral cortex membranes at 37°C . Two incubation tubes with a total volume of 1.5 ml were used. Concentrations of membranes and ^3H -(-)-alprenolol were as described in the text. One tube contained (-)-propranolol (1×10^{-5} M). After starting the binding reaction by adding membranes and stirring, 100 μl aliquots were removed from each tube at the times indicated. These aliquots were added to 5 ml of ice cold buffer and were kept at 4°C until filtered within 10 min. The tube was washed with an additional 5 ml of buffer. Binding dissociates less than 5% at 4°C in 10 min. At 10 minutes ($\pm$)-propranolol (100 μM) was added (arrow). The forward and reverse time curves represent the means of 3 and 2 experiments respectively.



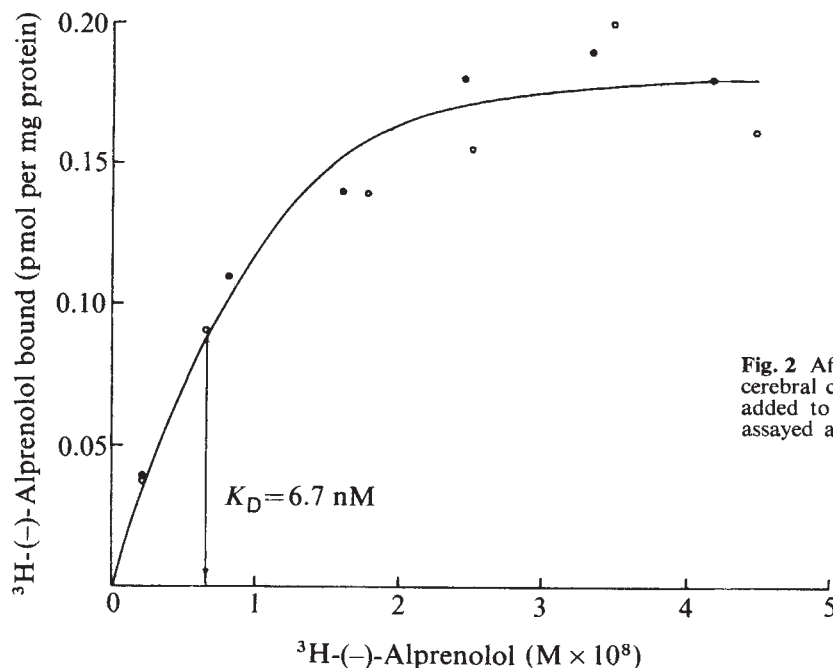


Fig. 2 Affinity and saturability of ^3H -(-)-alprenolol sites in rat cerebral cortex. Increasing amounts of ^3H -(-)-alprenolol were added to a fixed amount of membrane protein. Binding was assayed as described in the text. Two experiments are shown.

Prusoff¹⁴ and Rodbard and Lewald¹⁵ the EC_{50} values in Table 1 are approximately four- to tenfold higher than the actual K_D values. It should be stressed that as the receptor and ^3H -(-)-alprenolol concentrations in the assays decrease, the EC_{50} values for various ligands will also decrease and ultimately approach their true K_D values. Thus, the observed EC_{50} in the binding assay is only a relative measure of the affinity of an agent for the receptor which is influenced by a variety of technical factors. The K_D for any agent, which may be calculated from the EC_{50} (refs 14 and 15) is, however, a constant presumably independent of assay conditions.

As may be expected from their similarity to β -adrenergic agents (ethylamine side chain) dopamine and 5-hydroxytryptamine (5-HT) competed for ^3H -(-)-alprenolol binding sites at high concentrations. Catecholamine precursors or metabolites and octopamine compete weakly or not at all for the binding sites. Other putative neurotransmitters such as glycine, 4 aminobutyric acid, and histamine are only weakly effective or ineffective in competing for binding. Pyrocatechol does not compete for the binding sites. The α -adrenergic antagonist phentolamine

displaces 50% of specifically bound alprenolol at 1×10^{-4} M.

The distribution of ^3H -(-)-alprenolol binding sites is compared with the concentration of noradrenaline in different regions of the rat CNS in Table 2. The areas highest in binding sites, the cerebral cortex, limbic forebrain and corpus striatum, had concentrations of binding sites comparable with the number of binding sites in canine myocardial membranes, a tissue rich in β -adrenergic receptors⁵. The hypothalamus and adjacent diencephalon had high noradrenaline content but relatively fewer ^3H -(-)-alprenolol binding sites. The cerebellum and hippocampus, areas where β -adrenergic receptors have been identified electrophysiologically^{16,17}, have approximately the same proportion of binding sites and noradrenaline. In spite of substantial amounts of noradrenaline in the spinal cord and medulla, little specific ^3H -(-)-alprenolol binding was observed in these regions.

The ^3H -(-)-alprenolol binding sites in rat brain have characteristics similar to those described for β -adrenergic receptors in other tissues such as the heart⁵, frog erythrocytes⁴ and human lymphocytes¹⁸. Binding is rapid and reversible,

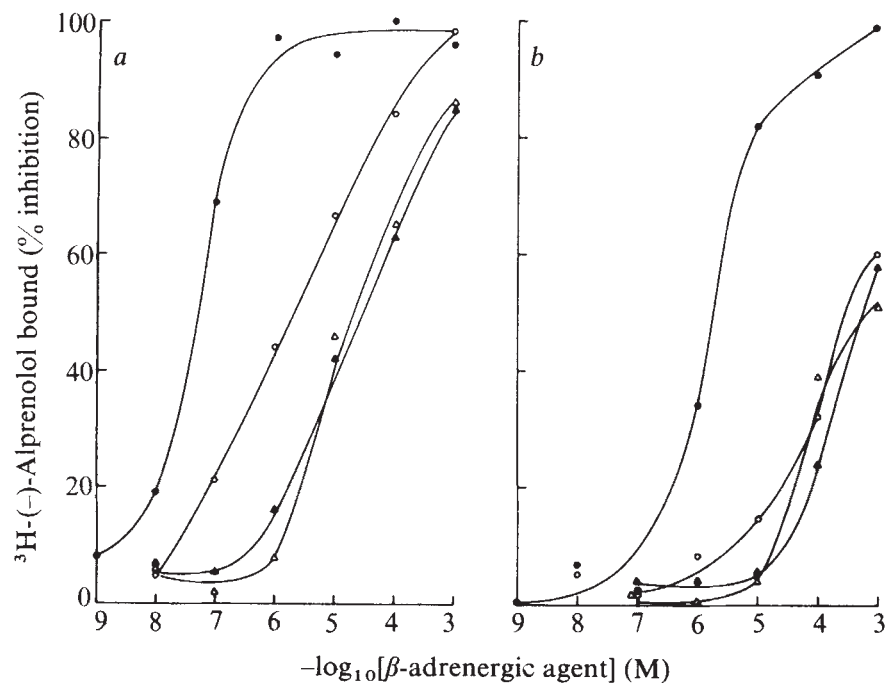


Fig. 3 Specificity of ^3H -(-)-alprenolol binding in rat cerebral cortex membranes. Dose-response curves for the inhibition of ^3H -(-)-alprenolol binding by the (-)-stereoisomers of propranolol ($\bullet$), isoproterenol ($\circ$), noradrenaline ($\blacktriangle$), and adrenaline ($\triangle$) are shown in *a*. The inhibition of ^3H -(-)-alprenolol binding by the (+)-stereoisomers of these agents is shown in *b*. The symbols are identical to those used in *a*.

stereospecific, saturable and exhibits high affinity for β -adrenergic antagonists. These features suggest that the binding sites in the present study represent physiological β -adrenergic receptors. The catecholamine potency series defines these receptors as being of the β_1 subtype¹⁹.

The ability of the α -adrenergic antagonist phentolamine to displace ^3H -(-)-alprenolol at high concentrations is compatible with the "nonspecific" inhibitory effects of high concentrations of phentolamine on β -adrenergic physiological responses²⁰ and on β -adrenergic receptor coupled adenylate cyclase in other tissues²¹. This nonspecific effect on the β -adrenergic receptor of rat brain suggests that the designation, based on phentolamine inhibition, of catecholamine effects in the rat brain as being α -adrenergic receptor-mediated be approached with caution.

The displacement of ^3H -(-)-alprenolol by 5-HT at 10^{-4} M is not surprising in view of the fact that 5-HT at high concentrations has been shown to inhibit catecholamine stimulation of β -adrenergic receptor coupled adenylate cyclase in avian erythrocytes²² and rat pineal²³ and to inhibit noradrenaline induced cyclic AMP accumulation in various areas of the rat brain²⁴. The present data do not permit a conclusion as to whether 5-HT interacts with the β -adrenergic receptor of rat cerebral cortex as an antagonist or perhaps as a partial agonist.

There was no apparent correlation between levels of noradrenaline and number of β -adrenergic receptors in various brain regions. Similar lack of correlation between levels of transmitter and number of receptors for that transmitter has been observed in other instances as has been reviewed²⁵. Possible explanations include variations in different brain regions of: (1) density of noradrenergic innervation of neurones; (2) noradrenaline content of nerve terminals; and (3) relative surface area of the postsynaptic membrane associated with noradrenergic nerve terminals. In addition, noradrenaline nerve terminals could be associated with α -adrenergic postsynaptic receptors.

Noradrenaline has an inhibitory effect on the discharge of Purkinje cells in the rat cerebellum^{10,11}. This inhibitory effect is mediated through a β -adrenergic receptor-adenylate cyclase system since the effects are mimicked by the iontophoretic application of cyclic AMP and since β -adrenergic antagonists block the effects of noradrenaline but not those of cyclic AMP. Inhibition of discharge rates by noradrenaline and cyclic AMP has also been observed in pyramidal tract neurones of the rat cerebral cortex¹².

Table 1 Effects of various agents on ^3H -(-)-alprenolol binding to rat cerebral cortex membranes

Agent	Half-maximum inhibition of ^3H -(-)-alprenolol binding (μM)
(-)-Propranolol	0.056
(+)-Propranolol	1.4
(-)-Isoproterenol	2.0
(-)-Noradrenaline	16.0
(-)-Adrenaline	16.0
Phentolamine	100.0
5-HT	100.0
(+)-Isoproterenol	440.0
(+)-Adrenaline	708.0
(+)-Noradrenaline	900.0
Dopamine	*
($\pm$)-Octopamine	*
($\pm$)-Normetanephrine	*
($\pm$)-3,4-dihydroxymandelic acid	NI
($\pm$)-Dihydroxyphenylalanine (dopa)	NI
Tyramine	NI
Pyrocatechol	NI
Glycine	NI
(-)-Aspartic acid	NI
(-)-Glutamic acid	NI
Histamine	NI
4-Aminobutyric acid	NI

^3H -(-)-alprenolol binding was assayed as described in the text. Maximum inhibition of binding is defined as the binding inhibited by 10^{-6} M (-)-propranolol. *Less than 50% inhibition of binding at 1×10^{-4} M; NI, no inhibition of binding at this concentration.

Table 2 Distribution of ^3H -(-)-alprenolol binding sites and noradrenaline in the rat CNS

Brain area	^3H -(-)-Alprenolol binding sites (pmol/mg protein)	Noradrenaline (ng/g frozen tissue)
Limbic forebrain	0.28 ± 0.06 (4)	772 ± 44 (3)
Hippocampus	0.26 ± 0.02 (4)	417 ± 28 (4)
Parietotemporal cortex	0.23 ± 0.02 (4)	350 ± 7 (3)
Corpus striatum	0.22 ± 0.02 (4)	162 ± 35 (4)
Frontal cortex	0.20 ± 0.03 (4)	428 ± 44 (4)
Diencephalon	0.20 ± 0.03 (4)	674 ± 66 (4)
Hypothalamus	0.15 ± 0.01 (4)	$1,652 \pm 145$ (4)
Pons	0.11 ± 0.01 (4)	649 ± 42 (4)
Cerebellum	0.11 ± 0.02 (4)	207 ± 18 (4)
Medulla	0.06 ± 0.02 (4)	342 ± 20 (4)
Spinal cord	0.06 ± 0.02 (6)	423 ± 41 (8)

Fresh rat brains were dissected on an iced Petri dish with fine forceps. The corpus callosum was split and the two cerebral cortices were played by opening the lateral ventricles. Using the anterior commissure as a landmark, the limbic forebrain (septal nuclei, nucleus accumbens and olfactory tubercles) was dissected from the diencephalon. The corpus striatum was next dissected and then the fornix was divided. The hippocampus, parietotemporal cortex, and frontal cortex were then separated. The cerebellum was removed from the remaining brainstem and the medulla was separated from the pons at the pontomedullary junction. The brainstem was inverted and the hypothalamus was removed by dissection of the grey matter surrounding the median eminence from the surrounding white matter. The pons was separated from the rest of the brainstem which was designated as the diencephalon. Each brain region was frozen on dry ice. For the noradrenaline assays, regions from three animals were pooled for each determination. ^3H -(-)-alprenolol binding sites were assayed by adding saturating levels of ^3H -(-)-alprenolol with or without 10^{-6} M (-)-propranolol to membranes and assaying as described in the text. Noradrenaline was assayed by a fluorometric method as described previously²⁶. Values represent mean $\pm$ s.e.m. followed by the number of experiments in parentheses. Each point was assayed in duplicate.

It is tempting to postulate that the binding studied in the present investigation represents postsynaptic β -adrenergic receptors involved in information transmission between neurones. Such a speculation requires further substantiating evidence. This is especially true in view of the fact that a β -adrenergic receptor coupled adenylate cyclase has been reported in various glial cell cultures^{26,27}. Thus, the anatomical localisation of the ^3H -(-)-alprenolol binding sites in the brain requires further investigation.

Direct binding with ^3H -(-)-alprenolol provides a new technique for studying the role of the β -adrenergic receptor in the neurophysiology and pathophysiology of the CNS. In view of the technical difficulty in maintaining catecholamine responsiveness of adenylate cyclase in brain homogenates¹³, these binding techniques may ultimately facilitate the localisation of sites of hormonal responsiveness in the CNS. The direct study of the regulation of the brain β -adrenergic receptor as a physiological entity is also now possible.

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Prolonged activation of tyrosine hydroxylase in noradrenergic neurones of rat brain by cholinergic stimulation

In sympathetic ganglia and adrenal medulla, the augmented release of acetylcholine from preganglionic sympathetic neurones can lead to prolonged changes in the activity of tyrosine hydroxylase (TH), the enzyme catalysing the rate-limiting step in the biosynthesis of catecholamines^{1,2}. The trans-synaptically mediated increase in TH activity is due to excitation of cholinergic receptors³⁻⁵, occurs after a latency of many hours to days, remains elevated up to weeks and is attributable entirely to increased accumulation of TH enzyme molecules; it therefore represents induction of the enzyme.

In brain, a three- to fourfold increase in the activity and amount of TH, comparable in many respects to the trans-synaptic induction of the enzyme observed in ganglia and adrenals, can be initiated in the cell bodies of noradrenergic neurones of the locus coeruleus by reserpine^{6,7}. Since the locus coeruleus contains large amounts of enzymes subserving the synthesis and degradation of acetylcholine^{8,9}, we wondered whether stimulation of cholinergic receptors would induce TH in this area of the brain as it does in the periphery.

We therefore set out to determine if the centrally active cholinergic agonist, oxotremorine, which stimulates muscarinic receptors¹⁰ can induce TH in the locus coeruleus. We report that the administration of oxotremorine to rats results in a prolonged elevation of TH activity in the locus coeruleus, similar in time course to that elicited by reserpine. The biochemical basis for the increase in enzyme activity is, however, entirely different from that produced by reserpine, being entirely attributable to prolonged increase of the catalytic activity of the enzyme molecules (activation). This mode of regulation of TH, which we have termed delayed activation, seems not to have been recognised before.

Oxotremorine (Aldrich Chemical Company, Inc.) was administered to male Sprague-Dawley rats weighing 225-275 g, in doses from 0.25 to 2.5 mg kg⁻¹ subcutaneously. Controls received saline. The animals were killed by cervical dislocation on various days thereafter, and the region of the locus coeruleus was dissected¹¹ and homogenised in 10 volumes of 0.005 M phosphate buffer containing 0.2% Triton X-100. Tyrosine hydroxylase was

assayed by a modification of the method of Coyle¹², with omission of quinonoid dihydropterin reductase and using mercaptoethanol for stabilisation of the cofactor DMPH₁ (6,7-dimethyl-5,6,7,8-tetrahydropteridine). Saturating concentrations of tyrosine and DMPH₁ were used. Enzyme activity was calculated as pmol dopa formed per h per pair of loci coerulei for each animal.

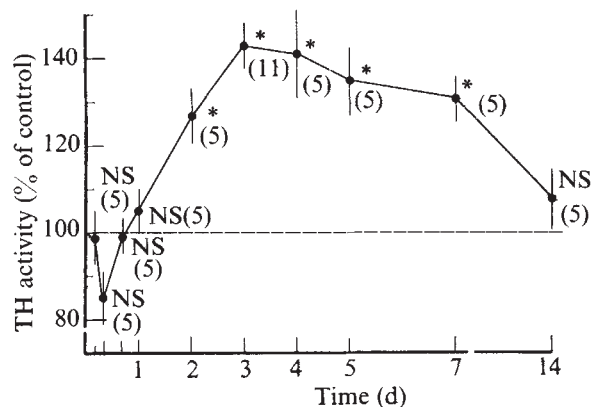
A single injection of oxotremorine (1.5 mg kg⁻¹) resulted in a prolonged increase in the activity of TH in the locus coeruleus (Fig. 1). The increased activity appeared after a latency of 24 h, rose to a maximum of 40% of control at 72 h after the injection and returned to control approximately 14 d later. The increase was dose dependent with a threshold response of 0.5 mg kg⁻¹, and seemed specifically related to activation of muscarinic cholinergic receptors since it was blocked by atropine (40 mg kg⁻¹, administered intraperitoneally 30 min before oxotremorine).

We next sought to determine by immunotitration with a specific antibody to TH^{6,13}, if the increase in enzyme activity in the locus coeruleus produced by oxotremorine was due to increased accumulation of enzyme molecules or to activation of the existing enzyme. Immunochemical titration was performed by our adaptation¹³ of the method of Feigelson and Greengard¹⁴, using specific antibodies to bovine adrenal TH prepared as described elsewhere^{6,13}. Two immunotitration procedures were used. In the first, increasing amounts of tissue from control and treated animals were added to a fixed amount of antibody, the antibody-enzyme complex was precipitated out, and enzyme activity remaining in the supernatant was determined. The concentration of tissue (enzyme) that precipitates out all the antibody is termed the equivalence point and is graphically identified as the point on the abscissa at which enzyme activity first appears in the supernatant.

In the second immunotitration procedure, the tissue extract of treated animals was diluted so that the enzyme activity in an aliquot was identical to that in an equal volume of homogenate from controls.

The results of the first experiment are illustrated in Fig. 2a and demonstrate that the immunotitration curves of the locus coeruleus of oxotremorine-treated animals was steeper than that of saline-injected controls, but shared an identical equivalence point. This observation indicates that the number of immunoreactive molecules of TH in the locus coeruleus in treated animals is the same as for controls; however, the catalytic activity of each enzyme

Fig. 1 Time course of the changes in tyrosine hydroxylase (TH) activity in the locus coeruleus of the rat after injection of oxotremorine (1.5 mg kg⁻¹ subcutaneously). Values (mean $\pm$ s.e.m.) are expressed as percentages of controls. Control values were (mean $\pm$ s.e.m.; $n = 13$) 1,627 $\pm$ 49 pmol of dopa per pair of locus coeruleus. Figures in brackets represent number of observations. * $P < 0.001$ compared with controls; NS, not significant.



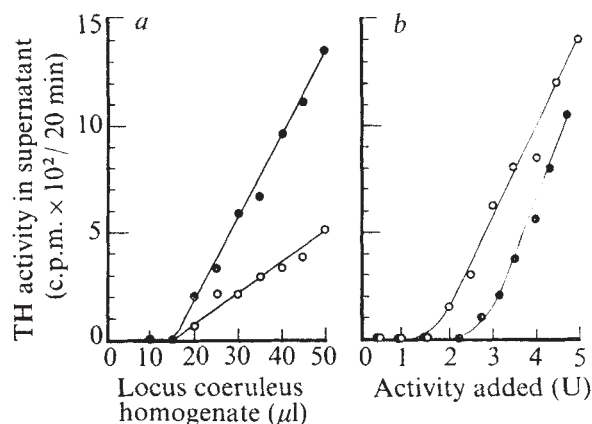


Fig. 2 Immunotitration of tyrosine hydroxylase (TH) in the locus coeruleus with an antibody to purified TH from bovine adrenal gland. Homogenates of brain tissue from the region of the locus coeruleus were prepared from rats treated with oxotremorine (1.5 mg kg⁻¹ subcutaneously once daily for 3 d; the rats were killed at d 6) or saline injected controls. ●, Oxotremorine; ○, control. *a*, A fixed amount of antibody (15 μl of antiserum) was added to test tubes containing increasing concentrations of supernatant (6,000g; 10 min) of tissue homogenate (0–50 μl in steps of 5 μl) diluted with homogenising buffer to give a constant volume of 50 μl in each tube. After incubation for 1 h at room temperature and subsequent centrifugation, TH activity was measured in the supernatant. Note that the same equivalence point was obtained for the oxotremorine- and saline-treated rats. *b*, Supernatants of locus coeruleus tissue homogenates from oxotremorine-treated rats were diluted with homogenising buffer and adjusted to give the same TH activity as in the supernatant of locus coeruleus tissue homogenate from control rats. Antiserum (15 μl) was added to test tubes containing increasing amounts of tissue extracts from the oxotremorine- and saline-treated groups respectively in a total volume of 50 μl. After incubation of the antibody-antigen mixture for 1 h at room temperature, the tubes were centrifuged and the TH was measured as the amount of ¹⁴C-dopa (c.p.m.) formed per 20 min per 50 μl of supernatant. Note that the oxotremorine treatment shifted the immunotitration curve to the right. The results indicate that oxotremorine caused an increase in activity of enzyme molecules rather than causing an accumulation of more enzyme protein.

molecule is increased. The second experiment (Fig. 2*b*) was confirmatory. Comparison of the immunotitration curves of homogenates of locus coeruleus of treated and control animals when adjusted for equal activity per unit volume, demonstrated that enzyme activity is increased without apparent increase in the amount of enzyme protein.

This study demonstrates that administration of the cholinergic agonist, oxotremorine, can increase the activity of tyrosine hydroxylase in central noradrenergic neurones of the locus coeruleus. The response occurs as a consequence of stimulation of central muscarinic receptors, either on locus coeruleus neurones or on interneurones, since it is blocked by atropine. The increase in TH activity elicited by oxotremorine is comparable in many respects to that produced by reserpine: it occurs after a latency of 24 h, reaches a peak (although smaller in magnitude) by 72 h and remains elevated for two weeks. But the immunochemical experiments show that, in contrast to reserpine, oxotremorine increases TH activity by activation rather than accumulation of enzyme molecules.

The delayed activation of TH by oxotremorine clearly differs from other modes of activation of the enzyme, such as those observed in terminals of locus coeruleus neurones by electrical stimulation¹⁵, or in terminals of central dopaminergic neurones, after lesions¹⁶ or administration of pharmacological agents including reserpine or neuroleptics¹⁷. These modes of activation have their onset within minutes of the stimulus, disappear within hours, and are associated with changes in the kinetics of the enzyme, either with respect to substrate^{15,17} or cofactor¹⁷. In contrast, the cholinergically activated enzyme not only

has a prolonged time course, but fails to demonstrate changes in the K_m for substrate or cofactor; an increased V_{max} being the only alteration in kinetics (T.H.J., T.L. and D.J.R., unpublished observations).

This cholinergically-mediated delayed activation of tyrosine hydroxylase therefore seems to represent a previously unrecognised mode of regulation of the enzyme. Its detection has been made possible only by the application of immunochemical techniques. The biochemical mechanisms underlying the changes in enzyme activity are unknown. The time course of the change is consistent with some process requiring the biosynthesis of macromolecules. One possibility would be that in response to the drug a different and catalytically more active form of the enzyme is synthesised; another mechanism, consistent with the kinetic data would be that a non-competitive inhibitor of the enzyme is removed. The fact that comparable activation of TH can be elicited by physostigmine (T.L., T.H.J. and D.J.R., unpublished), an agent which facilitates the action of naturally released acetylcholine by blocking its degradation, however, suggests that delayed activation of TH may be a naturally occurring mode of regulation of the enzyme. The functional significance of this mode of regulation and its possible role in mediating the action of centrally active agents acting on noradrenergic transmission remain to be established.

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Oestradiol receptor of neonatal mouse brain

ADMINISTRATION of oestrogen or androgen to neonatal rats or mice can cause sterilisation of females¹. Adult sterilised females generally exhibit masculinised behaviour and do not have normal cyclic gonadotropin patterns. Are prospective receptors for oestrogens and androgens present in neonatal rat or mouse brain tissues that are responsive to sex steroids? Direct demonstration of oestradiol receptor in brains of neonatal rats has been made difficult by the "neonatal binding protein" (NBP)^{2,3} that binds oestradiol with much higher capacity but lower affinity than does presumed receptor. If oestradiol receptor is present and functional, does NBP act in neonatal brain to prevent sterilisation by maternal oestrogens?³

Making use of the affinity for DNA of presumed oestradiol receptor from hypothalamus-preoptic area of pre-pubertal mice⁴, I have now separated this binding complex

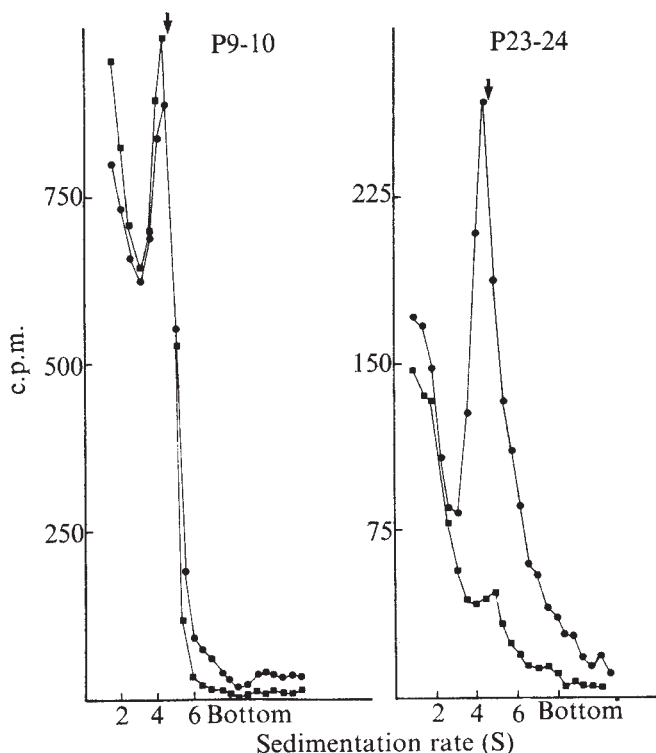


Fig. 1 Sucrose gradient sedimentation of bound ^3H -oestradiol incubated alone and with DES in extracts of hypothalamus-preoptic area from second and fourth week mice. As before^{1,4}, seven fourth week (postnatal days = P23-24) females (C57BL/6J $\times$ C3H/HeJ) F_1 hybrid mice were anaesthetised with ether, killed by saline perfusion and dissected to yield a block (wet weight, 0.30 g) of hypothalamus-preoptic area. The tissue was homogenised as described^{1,4} in buffer containing 0.01 M Tris-HCl, pH 8.1 (21 °C), 1 mM Na_3EDTA (ethylenediamine tetraacetate), 1 mM mercaptoethanol, 0.15 M NaCl and 10% (v/v) glycerol and then centrifuged to obtain a clear supernatant. Seven second week (postnatal days = P9-10) female F_1 hybrid mice were etherised, decapitated and dissected (tissue wet weight, 0.45 g). Young animals in this and the following experiments were treated in this manner to optimise presumed receptor yield by minimising preparation time. Aliquots of each extract were incubated with 4.5 nM ^3H -oestradiol (110 Ci mmol⁻¹, New England Nuclear) alone (●) or with 300 nM ($\times 67$) DES (■). Labelled extracts were freed of unbound oestradiol on Sephadex G-25 columns and then sedimented in 5-20% (w/v) sucrose gradients. The arrow indicates the internal fluorescent marker, dansyl-bovine serum albumin (sedimentation rate 4.7S).

from extracts of mice 2-12 d old. This fractionation directly demonstrates the existence of adult-like oestradiol receptor in neonatal mouse brain and permits examination of its binding characteristics in the presence of NBP. This technique enables me to test the suggested function of NBP and will facilitate quantitative studies of the separated receptor itself.

Initially oestradiol receptor was distinguished from NBP by the high affinity of the former for diethylstilboestrol (DES)^{2,3} (Fig. 1). In extracts of hypothalamus-preoptic area from 4-week-old mice (postnatal days 23-24), oestradiol binding to macromolecules sedimenting at 4S and faster was decreased more than 90% by excess non-radioactive DES. For 2-week-old mice (postnatal days 9-10), total oestradiol binding was higher, but was decreased only 20% by DES. As reported before^{1,4}, and usually seen for uterine receptor⁴, prepubertal brain receptor and brain NBP both sediment (Fig. 1) at about 4S in sucrose gradients containing 0.15 M NaCl, which was present to permit subsequent DNA-cellulose chromatography. Without NaCl (not shown), prepubertal receptor and NBP have different

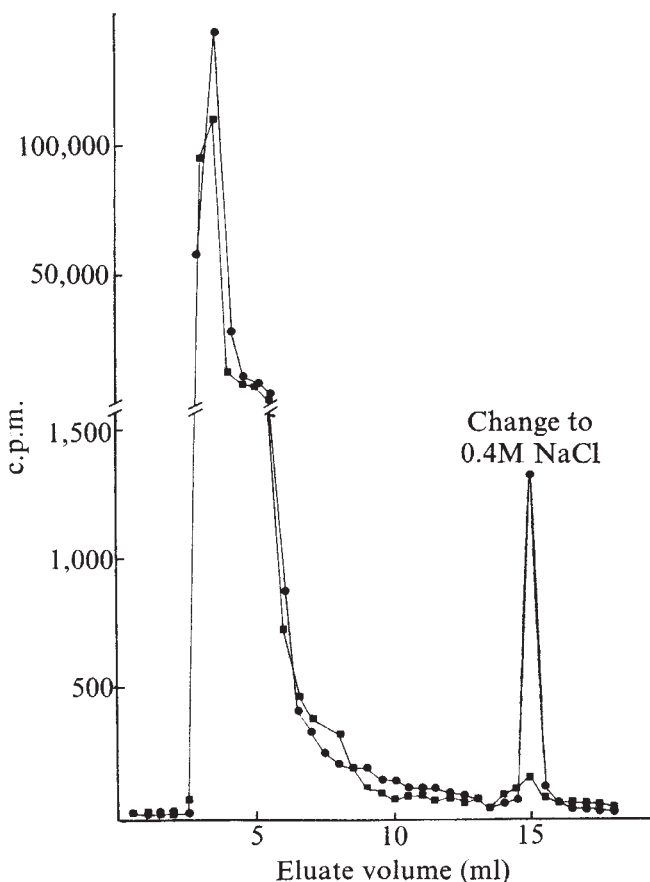
sedimentation rates^{1,4}; receptor sediments at 5.0-7.5S but NBP always has a sharp 4S sedimentation rate.

I do not believe that blood contamination accounts for the detected low affinity oestradiol binding (NBP). Preliminary experiments indicated that saline perfusion did not decrease detection of NBP, and blood pigment was not detected visually in extracts of hypothalamus-preoptic area from young mice. As suggested for young rats³, however, NBP may be derived from blood.

Earlier experiments with DNA-cellulose chromatography⁴ showed that up to half the presumed oestradiol receptor from prepubertal mouse hypothalamus-preoptic area bound specifically to DNA, while NBP from rat³ and mouse⁴ did not. DNA-cellulose chromatography was performed with extracts from 2-week-old mice (postnatal days 9-12) incubated with ^3H -oestradiol (Fig. 2). A portion of bound oestradiol did adhere to DNA-cellulose, and this was reduced 90% in the presence of non-radioactive DES. Together with the data in Fig. 1, the DNA-bound, DES-blocked fraction of bound oestradiol can be presumed to be adult receptor, separated from NBP. DNA-cellulose chromatography provides both purification and concentration of oestradiol receptor (Fig. 2, Fig. 3b and ref. 4). Reproducibly, almost all the eluted radioactivity can be collected in a single 0.5-ml vial.

Separation of the two oestradiol-binding macromolecules by DNA-cellulose chromatography allows comparison of their relative affinities for oestradiol. I have found¹ that

Fig. 2 DNA-cellulose chromatography of second week brain extracts with bound ^3H -oestradiol alone and with DES. An extract (wet weight 1.27 g) of hypothalamus-preoptic area from 22 female F_1 hybrids 9-12 d old was prepared in buffer with 0.15 M NaCl, as for the young animals described in Fig. 1. Aliquots were incubated with 4.8 nM ^3H -oestradiol alone (●) or combined with 320 nM DES (■). The complete labelled extracts were chromatographed on DNA-cellulose columns (1.5 ml packed volume) as described¹, except that carrier protein was not present in the buffers.



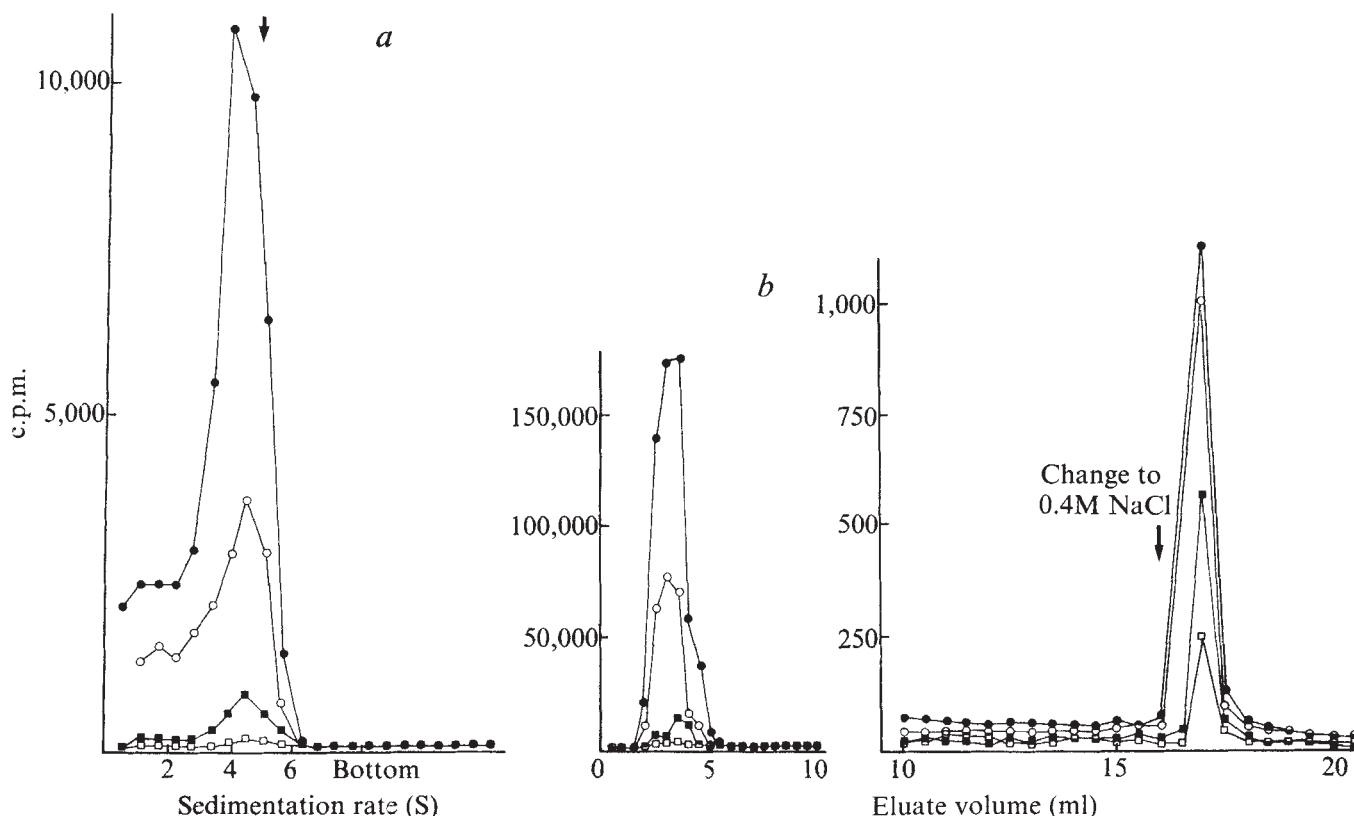


Fig. 3 Sucrose gradient sedimentation and DNA-cellulose chromatography of first week mouse brain extracts incubated with different concentrations of ^3H -oestradiol. Extract (wet weight, 1.93 g) was prepared with hypothalamus-preoptic area from 33 female and male F_1 hybrids 3–7 d old. In an experiment comparing 2–3-d-old females and males, similar amounts of presumed oestradiol receptor were detected by DNA-cellulose chromatography, justifying the combination of females and males for this experiment. Aliquots were incubated with 1.4 nM ^3H -oestradiol ($\square$); 7.2 nM ^3H -oestradiol ($\blacksquare$); 36 nM ^3H -oestradiol ($\circ$); or 140 nM ^3H -oestradiol ($\bullet$). Each labelled extract was freed of unbound oestradiol on a Sephadex G-25 column^{1,4}. *a*, Aliquots of the Sephadex breakthrough fraction were sedimented on sucrose gradients to demonstrate that this fraction was predominantly bound to macromolecules at each concentration. The arrow indicated dansyl-bovine serum albumin (4.7S). *b*, DNA-cellulose chromatography of macromolecule-bound oestradiol. The abscissa has been split to allow two ordinates for non-DNA-cellulose-adhering NBP (eluting between 2 and 5 ml) and oestradiol receptor (eluting between 16 and 18 ml).

oestradiol receptor from prepubertal mouse hypothalamus-preoptic area is saturated by oestradiol at 4–8 nM. This is consistent with reported values⁹ of 10^{-9} – 10^{-10} M for the dissociation constant of adult oestradiol receptor from rat hypothalamus and related brain regions. In contrast, the dissociation constant for rat NBP is reported³ at 1.2×10^{-8} M. Since I have found (not shown) that NBP, present in large amounts in young mice, binds most of the oestradiol in solution in the range 1–140 nM, oestradiol receptor in its presence should have an apparent dissociation constant higher than normal. Accordingly, the DNA-adherent portion of bound oestradiol from young animals (as Fig. 2) should saturate at a higher concentration than usual if it is truly oestradiol receptor.

Oestradiol binding to oestradiol receptor and to NBP was compared for 1-week-old mice (postnatal days 3–7) after incubation with several concentrations of oestradiol (Fig. 3*b*). At higher oestradiol concentrations, total binding to NBP was greater (Fig. 3*a* and left portion in Fig. 3*b*), approaching saturation at 140 nM. Oestradiol binding to presumed receptor, however, increased only about threefold between 7.2 nM and 140 nM, nearly a twentyfold concentration range. Therefore, saturation of receptor was appreciably lower than for NBP, but 2–4 times greater than observed in pre-pubertal extracts. In the same experiment performed with 4-week-old mice (not shown), maximal DNA-binding was attained at 6 nM oestradiol. With 2-d-old mice (not shown), the same qualitative results were obtained, except with more NBP than in 1 and 2-week-old animals. Thus young mouse brain contains an appreciable amount of oestradiol receptor, but its quantita-

tive detection requires larger concentrations of ^3H -oestradiol than required for extracts of older animals.

Previously, receptor proteins for oestradiol have been found in the hypothalamus from adult and prepubertal rats^{2–3,5–6} and mice^{1,4}. Testosterone-binding proteins in rats^{7–10} and mice¹ have also been reported. Autoradiographic studies with 2-d-old rats indicate accumulation of ^3H -oestradiol and ^3H -testosterone by specific regions of brain¹¹. Quantitative binding studies with brain extracts from 5-d-old rats have suggested the presence of high affinity oestradiol-binding proteins¹², although regional specificity was not observed, and a small amount of presumed oestradiol receptor has been detected by sucrose gradient sedimentation of ^3H -oestradiol-labelled extracts from 7-d-old rats⁶.

The experiments described here show that extracts of hypothalamus-preoptic area from neonatal to 2-week-old mice contain macromolecules that bind oestradiol with high affinity and that can be distinguished from NBP by their affinity for DNA and their sensitivity to DES. These properties suggest that these macromolecules are adult-like oestradiol receptors. They are present in mice less than 1 week old, within the critical period, 7–10 d after birth, when oestrogens and androgens administered to mice or rats can alter sexual differentiation permanently^{13,14}. High affinity testosterone binding, in addition to oestradiol binding, has been detected in hypothalamus-preoptic area of 2-d-old mice^{1,15}.

The observation that NBP competes with presumed receptor for oestradiol supports the suggestion³ that this protein may be present in neonatal brain to protect it from

high levels of maternal oestrogen. They might otherwise oestrogenise all young mice and rats, causing females to be sterile¹³. DNA-cellulose separation methods make it possible to test this hypothesis quantitatively. Finally, these results elicit the caution that at least three factors must be included in equations of oestrogen reception in young rodent brain: oestrogen levels, receptor levels and levels of competing binding macromolecules. In other receptor systems, specific competing macromolecules might also need to be considered.

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Distinction between nerve growth factor and glial factor

THE morphogenesis of individual cell types can be studied in tissue culture: for example neuroblastoma cells can be induced to differentiate by a macromolecular factor released by cultured glioma cells¹. On the other hand, survival and process formation in cell cultures of dissociated ganglia from embryonic chicks, newborn mice or rats require the presence of nerve growth factor^{2,6}. An enriched population of non-neuronal cells can replace NGF in these systems and their effect can be impaired by addition of anti-NGF antibody^{2,3}. The fact that NGF has been detected in the homogenate of solid tumours induced by subcutaneous injection of glioma cells previously grown in culture, and that a partially purified fraction of this homogenate induces morphological differentiation in neuroblastoma cells⁴ suggests that NGF and glial factor are the same molecule. We present evidence that NGF and glial factor are molecules with different properties and distinct specificities.

The respective biological activities of NGF and glial factor have been investigated in cultures of chick dorsal root ganglia⁵, in dissociated cells cultured from rat superior cervical ganglia⁶ and in mouse neuroblastoma cell cultures¹. We confirmed that 2.5S NGF induces process formation in cultures of chick dorsal root ganglia⁵. In this system, NGF 0.004 $\mu\text{g ml}^{-1}$ and 0.016 $\mu\text{g ml}^{-1}$ gave responses + and + + + +, respectively (responses defined by Fenton⁸). In dissociated cells cultured from the rat superior cervical ganglia⁶ 0.125 $\mu\text{g ml}^{-1}$ was the lowest concentration at which an effect of 2.5S NGF could be detected, and 1 $\mu\text{g ml}^{-1}$ was the concentration routinely used. We found, however, that 2.5S NGF, tested at concentrations of 0.05, 0.1, 0.5, 1.0 and 10 $\mu\text{g ml}^{-1}$, does not induce morphological differentiation in neuroblastoma cells. In contrast the glial

factor, which causes morphological differentiation of neuroblastoma cells, had no effect in the two other biological assays. The different amounts of glia-conditioned medium or concentrated serum-free medium¹ tested in these bioassays represented 0.5, 1 or 10 times the glial factor activity necessary to obtain optimal morphological differentiation of neuroblastoma cells. These results indicate that the two factors have distinct specificities.

We further distinguished the factors by immunological methods. Purified anti-NGF antibody (K. S., C. Gagnon and H. T., in preparation), able to prevent NGF-induced process formation in the chick dorsal root ganglia and in the dissociated cell cultures of the rat superior cervical ganglia, does not interfere with the effect of glial factor in the neuroblastoma cell system. Table 1 shows that different concentrations of this purified antibody could not inhibit the morphological differentiation induced by the glia-conditioned medium. The lack of immunological cross reactivity indicates that the two factors are distinct molecules.

Finally, NGF and a partially purified preparation of glial factor were compared on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Fig. 1). In the gel in which the partially purified preparation of glial factor had been electrophoresed, not even a faint trace of staining could be detected where the 2.5S NGF subunit migrates. Assuming that we could have detected 0.2 μg of NGF in this system and knowing that we applied 738 U of glial factor activity, then 0.27 ng of NGF could be maximally present per unit of glial factor. Since it takes 10 U per 5 ml of culture medium to detect glial factor activity, the concentration of NGF present would be 20 times lower than the minimal concentration detectable in the chick dorsal root ganglia assay.

We conclude that NGF and glial factor are different molecules

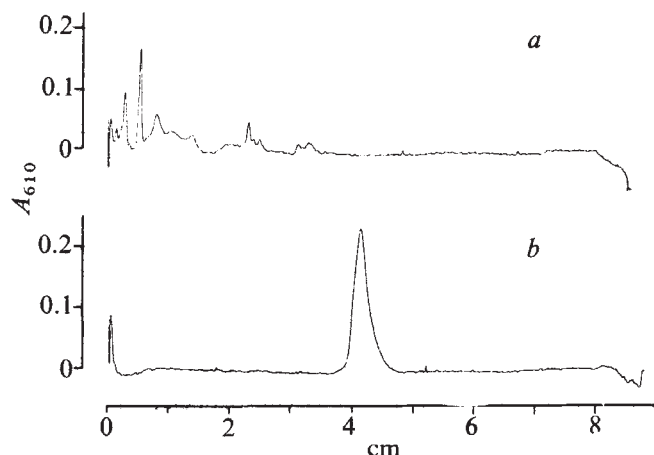


Fig. 1 Electrophoretic comparison of NGF and glial factor. C-6 glial cells were grown in rollers in Dulbecco's modified Eagles' medium supplemented with 10% foetal calf serum (Colorado Serum Co). After 72 h the medium was changed and the cells were incubated for 24 h in the same medium without foetal calf serum. This serum-free conditioned medium was concentrated 20-fold with a UM-10 Diaflo membrane and dialysed against phosphate buffer (6.7 mM $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 2.86 mM KH_2PO_4 , pH 7.35). The material was then loaded on a Whatman DE 52 cellulose column equilibrated with this buffer and submitted to stepwise elution with 0.2 M and 0.6 M NaCl in the same buffer. The glial factor activity is eluted in a very sharp peak just after the switch to the buffer containing 0.6 M NaCl. The main fraction of the peak had 3 μg of protein and contained 51% of the activity loaded on the column. This step of purification usually gives up to a 60-fold increase in the glial factor specific activity. This fraction was dialysed against double distilled water, lyophilised and resuspended in the sample buffer of the electrophoretic system⁹⁻¹⁰. The 15% polyacrylamide gels were stained with 0.1% Coomassie Blue R250 in 50% methanol and 7.5% acetic acid. After destaining with the same solvent mixture, they were scanned at 610 nm using a Zeiss PMQ II spectrophotometer. *a*, Partially purified glial factor; *b*, 20 μg of NGF purified by the method of Bocchini and Angeletti¹¹.

Table 1 Constancy of glia-induced morphological differentiation of neuroblastoma cells in the presence of different concentrations of anti-NGF antibody

Medium	μg Anti-NGF per ml	Cells with processes (%)
Conditioned	0	77.6 $\pm$ 4.7
Conditioned	0.1	75.6 $\pm$ 3.4
Conditioned	1.0	76.0 $\pm$ 4.9
Conditioned	10.0	74.8 $\pm$ 3.5
Normal	0	5.9 $\pm$ 0.8
Normal	10.0	5.9 $\pm$ 2.3

The induction and determination of morphological differentiation was carried out as described previously¹. The purified anti-NGF added to these cultures completely inhibited process formation in the chick dorsal root ganglia bioassay at a concentration of 0.1 $\mu\text{g ml}^{-1}$.

with distinct specificities. The action on the morphological differentiation of neuroblastoma cells observed by Longo and Penhoet⁴ with a partially purified NGF fraction from a solid tumour of glial origin could be explained by contamination with glial factor. Furthermore, as Levi-Montalcini⁷ pointed out, it is not yet certain that the NGF produced in the solid tumour is of glial origin.

Since glial factor acts on primary cultures of dissociated cells from embryonic rat brain (Y. Schürch-Rathgeb, and D. M., in preparation) and not on similar cultures of the superior cervical ganglia, we propose that glial factor and NGF produce similar effects but on different target cells. Further studies are required to establish the sites of action, the specificity and the possible morphogenic implications of the glial factor in the central nervous system.

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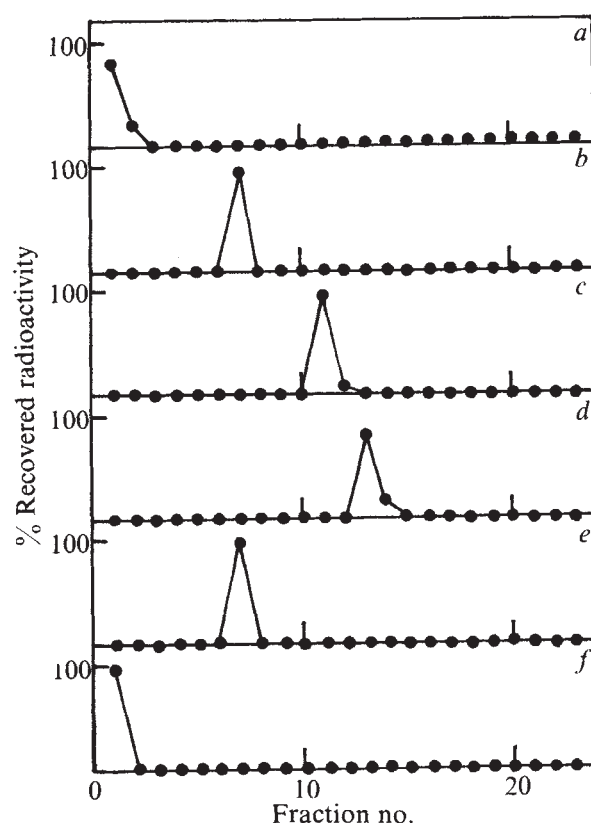


Fig. 1 Effect of actinomycin D on the sedimentation of a DNA-protein complex. FM3A cells established from mouse mammary carcinoma²⁰ were cultured in suspension as before¹⁸. Cells labelled with ¹⁴C-thymidine (62 mCi mmol⁻¹) at 0.05 $\mu\text{Ci ml}^{-1}$ for 24 h were treated at a density of 3×10^5 cells per ml with actinomycin D (Merck, Sharp and Dohme) for 30 min at 37 °C at various concentrations (a, 0; b, 1; c, 3; d, 10; e, 70; f, 100 $\mu\text{g ml}^{-1}$). DNA-protein complex released by lysing the cells in 1.3% SDS solution on top of a neutral sucrose gradient was spun at 36,000g for 60 min at 20 °C (ref. 18). After centrifugation, fractions were collected from the bottom of the tube and acid-precipitable radioactivity was counted in Beckman scintillation spectrophotometer.

supercoiled and folded by a few non-histone proteins tightly bound to it.

When FM3A cells labelled with ¹⁴C-thymidine were analysed by sodium dodecyl sulphate (SDS)-neutral sucrose gradient centrifugation, DNA sedimented as a complex with proteins containing disulphides^{18–20}. When cells were treated with various concentrations of actinomycin D and analysed in the same way, the sedimentation rate of the complex varied depending on the concentration of the agent used (Figs 1 and 2). As Fig. 2 shows, the amount of actinomycin D bound to DNA increased proportionately as the concentration of the drug added to the culture medium was increased up to 70 $\mu\text{g ml}^{-1}$. The minimum sedimentation rate of the complex was obtained at a concentration of about 20 $\mu\text{g ml}^{-1}$. Above or below this concentration sedimentation rates were larger. The sedimentation rate of the complex from untreated control cells was reduced if it was treated with Pronase known to be free of DNase activity in the top layer of the gradient^{18,19} (Fig. 2). Trypsin and 2-mercaptoethanol caused the same reduction in the sedimentation rate of the complex as did Pronase^{19,20}, while RNase A and T1 and phospholipase A and C had no effect (ref. 19 and our unpublished data). The Pronase-digested DNA-protein complex from actinomycin-treated cells showed a similar but less pronounced transition in the sedimentation rate with a minimum value at 20 $\mu\text{g ml}^{-1}$. When cells were irradiated with γ rays before treatment with actinomycin

Supercoiled DNA folded by non-histone proteins in cultured mammalian cells

A LONG-CHAIN DNA molecule must be highly folded within the nucleus of the eukaryotic cell with some regularity. Information about the structure of chromatin has come from X-ray diffraction^{1–4}, physicochemical studies^{5,6}, electron microscopy^{7–11} and nuclease digestion^{12–15}, and suggested that chromatin from eukaryotes comprises a linear array of spherical particles in which DNA is supercoiled around a core of oligomers of histones^{16,17}. We have used another approach to assess the structure of DNA in the nucleus of cultured mammalian cells, and have evidence suggesting that when all the histones and most of the non-histone proteins are removed from chromatin, DNA is

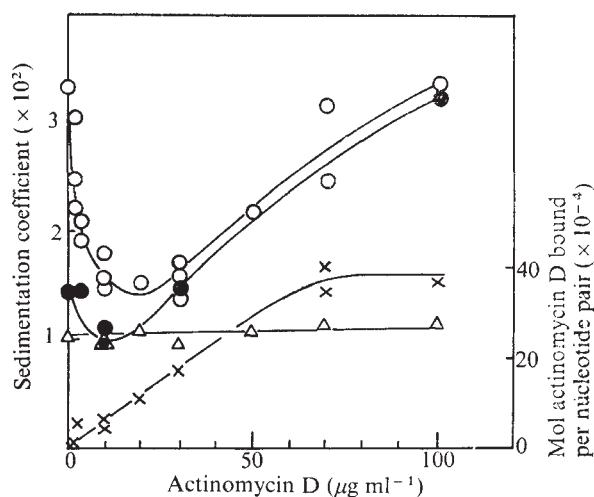


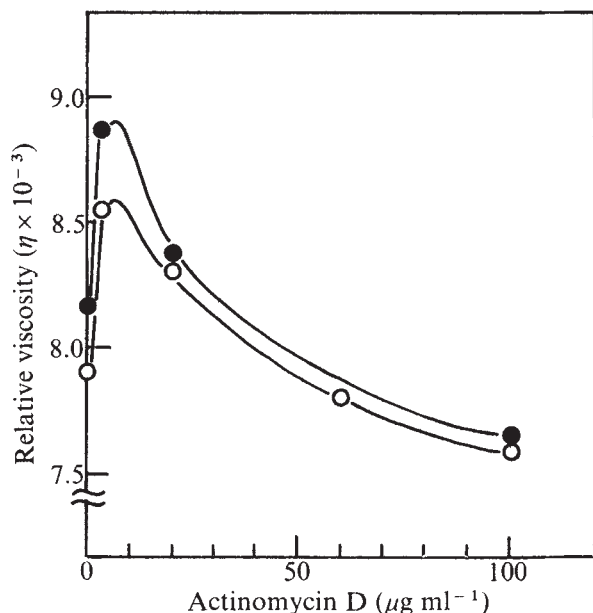
Fig. 2 Effect of actinomycin D and Pronase on the sedimentation of DNA-protein complex. FM3A cells were treated with actinomycin D and lysed in SDS on top of the gradient with (●) or without (○) Pronase E (1.5 mg ml⁻¹). Cells irradiated with γ rays at a dose of 24,000 r from a ⁶⁰Co source and treated with actinomycin D (Δ). Sedimentation coefficient was calculated with ³H- λ_{c1} particles as a reference marker. The amount of actinomycin D bound per nucleotide pair of DNA calculated using ³H-actinomycin D bound in the DNA fraction was the same whether cells were irradiated or not, and so those from control cells were plotted (x).

D the characteristic biphasic transition was abolished.

The viscometric behaviour of the DNA-protein complex was the opposite to that in sedimentation analysis (Fig. 3). The relative viscosity of the complex increased initially to a maximum and then decreased through continued binding of actinomycin D. Relative viscosity increased when the complex was treated with Pronase.

A large quantity of the DNA-protein complex was collected from scaled up gradients, and the proteins contained in it were dissociated from DNA with 2-mercaptoethanol and analysed by SDS-polyacrylamide gel electro-

Fig. 3 Effect of actinomycin D and Pronase on the viscosity of the DNA-protein complex. FM3A cells (5×10^5 cells per ml) were treated with various concentrations of actinomycin D for 30 min at 37 °C and lysed by mixing with the same volume of 2% SDS with (●) or without (○) Pronase E (1.5 mg ml⁻¹) in the chamber of a capillary type Ostwald viscometer. Relative viscosity was calculated with water as a reference standard.



phoresis. As Fig. 4 shows, two non-histone proteins with apparent molecular weights of approximately 70,000 and 55,000 were obtained.

Taken together our experiments suggested a basic structure of DNA in the nucleus of the mammalian cell. (1) DNA is supercoiled and folded by a few non-histone proteins. (2) Dissociation of the proteins from DNA releases folds in DNA, but the DNA remains supercoiled and thus folds and supercoils maintain the compactness of the DNA-protein complex which has a large sedimentation rate and a small relative viscosity. (3) Unwinding of supercoils by intercalating agents and/or unfolding after the disruption of protein cores converts the DNA into a relaxed structure with a small sedimentation rate and a large relative viscosity. The minimum sedimentation rate and the maximum relative viscosity in folded and unfolded DNA are obtained when all superhelical turns are lost. Continued binding of the intercalating agents results

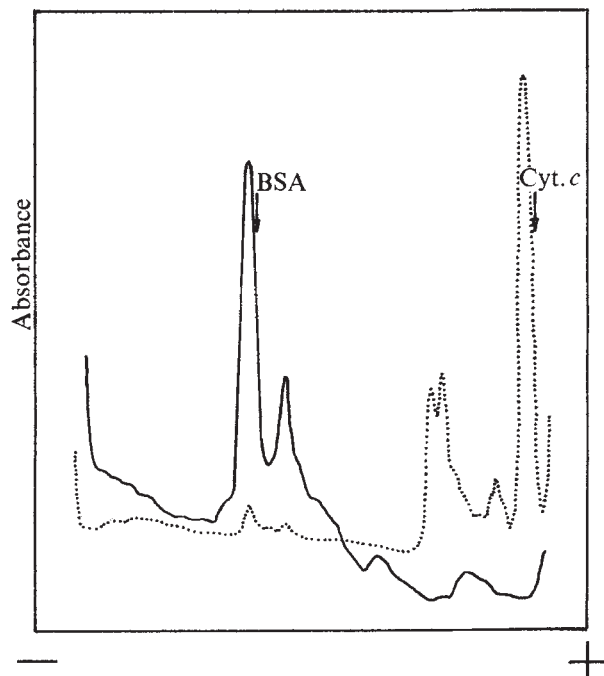


Fig. 4 Spectrophotometric scan of Coomassie blue-stained proteins contained in the DNA-protein complex after electrophoretic separation in an SDS-polyacrylamide gel. DNA-protein complex collected from the sucrose gradients was sonicated and concentrated. Then proteins were dissociated by the treatment with 2-mercaptoethanol and electrophoresed in an SDS-polyacrylamide gel column (—). Purified whole histone fractions from calf thymus (....), bovine serum albumin and cytochrome *c* (arrows) were electrophoresed in parallel gels as reference standards. The direction of electrophoresis was from left to right.

in the formation of superhelical turns of opposite handedness, and thus an increase in sedimentation rate and a decrease in relative viscosity. Further support for this contention comes from two sources. First, results similar to those described here with actinomycin D were obtained with other intercalating agents such as acriflavin and ethidium bromide. Second, in a microscopic autoradiogram of the DNA-protein complex spread on a Millipore membrane, the slow sedimenting complex was shown to be a relaxed mass of DNA fibres in contrast to a compact mass of DNA from the fast sedimenting complex.

The supercoiled structure of DNA suggested here is for the DNA-protein complex obtained by dissociation of all the histones and most of the non-histone proteins from chromatin, and thus may have derived from the supercoils localised in the unit spherical particles of chromatin. A similar structural model of DNA in higher organisms has

been proposed^{12,22} in which DNA is folded into loops held by some proteins.

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Nucleosome formation abolishes base-specific binding of histones

CHROMATIN has a subunit structure¹⁻³ and each subunit, for which the term nucleosome has been suggested⁴, has been proposed to consist of two of each of the four histones H2A, H2B, H3 and H4, and 200 base pairs of DNA (ref. 3). Do the histones responsible for generating the nucleosomes bind to specific sequences of the DNA? Heterologous reconstitution experiments showed that the four histones bound to DNA from bacteria or viruses gave rise to the same X-ray diffraction pattern⁵, the same size of nuclease-resistant DNA fragments⁶, and the same subunit structure, shown by electron microscopy⁷, as obtained for native chromatin. These findings suggest that the nucleosomes do not contain specific DNA sequences. This agrees with the apparently random distribution of nucleosomes along adenovirus DNA⁸. On the other hand, there is evidence that individual histones and polypeptides like polylysine or polyarginine bind preferentially to certain DNA sequences⁹⁻¹⁰.

We have approached the question of the specificity of binding of histones to DNA sequences in the following way: λ dv21 DNA, a small plasmid of molecular weight 2.1×10^6 , was linearised by digestion with restriction nuclease HindIII, and λ dv21 histone complexes were reconstituted with calf thymus histones. The location of histones on the linear DNA was then investigated using restriction nuclease HindII which cleaves λ dv21 DNA at five sites (Fig. 1). The individual histones as well as the tetramer complex (H3)₂ (H4)₂ (refs 12, 13) all protected two adjacent HindII sites from cleavage much better than the other three, indicating specificity of binding. This specificity was not found when complexes reconstituted with the four histones which constitute the nucleosome (that is H2A, H2B, H3 and H4) were cleaved with the restriction enzyme.

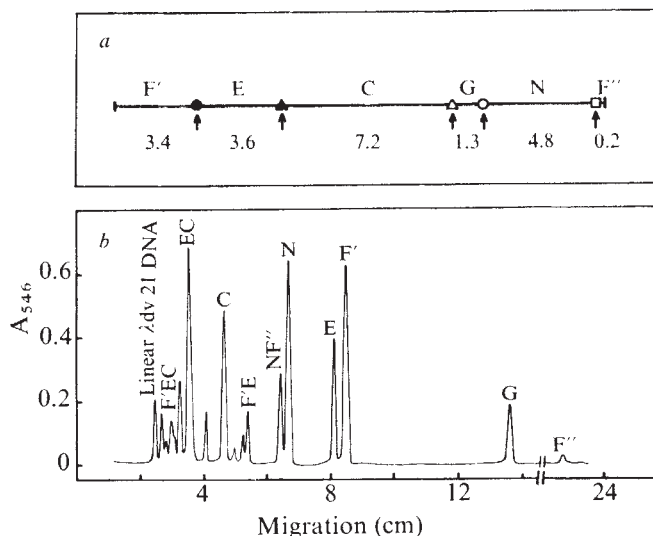
Figure 2 shows HindII digestion patterns of complexes of linear λ dv21 DNA with increasing amounts of histones. Some λ dv21 cleavage sites were protected by histones, for partial digestion fragments appeared in conditions just sufficient for complete cleavage of the free DNA. In more extensive digestion conditions complete cleavage of the complexes could be achieved (not shown). Thus the protection of cleavage sites by histones can be overcome by restriction nuclease. To evaluate the protection of cleavage sites quantitatively, we used densitometry of polyacrylamide gels. A densitogram of a partial digest of λ dv21 DNA is shown in Fig. 1b. As Fig. 3 shows, all the histones, when tested individually, protected the two cleavage sites on the left of the map (between F', E, and C) better than the three other sites. In the 100% H2A complex the fragments C, E and F' were missing and fragment F'EC appeared instead. A similar specificity was found with H1 and H2B and to a lesser extent with H3 and H4.

The extent of protection differed for the individual histones. H1 protected all five cleavage sites very efficiently. Polylysine also protected the DNA against the nuclease quite efficiently, but it did not show the same specificity as did the histones. Instead, it had slight preference for the left cleavage site between E and C and the right one between N and F'. Polyarginine behaved much like polylysine (not shown).

Preferential protection by histones is not unique to cleavage sites for HindII. We also found preferential protection of cleavage sites for *Bacillus subtilis* restriction nuclease which were close to the HindII cleavage site between F' and E (Fig. 1). The preferential protection of the left-hand HindII cleavage sites may be due to preferential binding of the individual histones to particular DNA sequences at or near the cleavage sites. The protected HindII site between F' and E lies in the rightward operator and promoter region of phage λ , which has been sequenced and found to consist of alternative highly AT-rich and highly GC-rich DNA sequences, each about twelve nucleotides long²⁰. This microheterogeneity might favour preferential binding of individual histones to this region.

It should be noted that the specificity of histone binding

Fig. 1 HindII digestion of linear λ dv21 DNA. *a*, Cleavage map of linear λ dv21 DNA. The HindIII site is located in fragment F, giving rise to fragments F' and F''. Molecular weights ($\times 10^{-5}$) of the fragments are indicated¹¹. *b*, Densitogram of a gel electrophoretic separation of fragments from a partial HindII digestion of linear λ dv21 DNA. For experimental details see legend of Fig. 2. The assignment of bands to particular HindII fragments was based on the determination of molecular weights which were compared with those calculated from the cleavage map. Some fragments are indicated.



depended on the salt concentration. When histone DNA complexes were digested which had been dialysed against 100 mM NaCl in buffer in the last reconstruction step, no specificity was observed.

The tetramer complex $(H3)_2(H4)_2$ which was postulated³ to be the core of the nucleosomes clearly showed the same specificity of protection as the individual histones (Figs 2b and 3). Protection of the two adjacent cleavage sites by the tetramer complex was more specific than that by H3 and H4 tested individually. The specificity of protection was lost, however, when λ dv21 DNA was complexed with an equimolar mixture of the four histones which constitute the nucleosome: all five cleavage sites were protected to the same extent (Fig 3). The very slight specificity of protection observed in the 40% complex may be due to incomplete nucleosome formation. Fragment patterns very similar to those of Fig. 2c could also be produced by partial digestion of histone-free linear λ dv21 DNA (for example Fig. 1b) while those of Fig. 2a and b were unique to the histone com-

plexes. This also demonstrated that the protection of cleavage sites in the presence of the four nucleosome-forming histones was unspecific. When an equimolar amount of H1, in addition to the four histones, was included in the reconstitution mixture, the protection of all five cleavage sites was more efficient than without H1, but again there was preferential protection of the two cleavage sites on the left side of the map (Fig. 3).

To determine whether nucleosomes had been formed in our reconstitution procedures we did digestion experiments with micrococcal nuclease. Degradation of the complexes between DNA and the four histones gave rise to discrete DNA fragments of about 180 and 360 base pairs, similar in size to those obtained from native chromatin⁴. When reconstitution was carried out in the absence of urea^{12,21,22}, DNA fragments of threefold unit length were also detected on digestion with micrococcal nuclease. More than 80% of the DNA could be converted into the monomer band when the 100% complex which had been formed in the absence

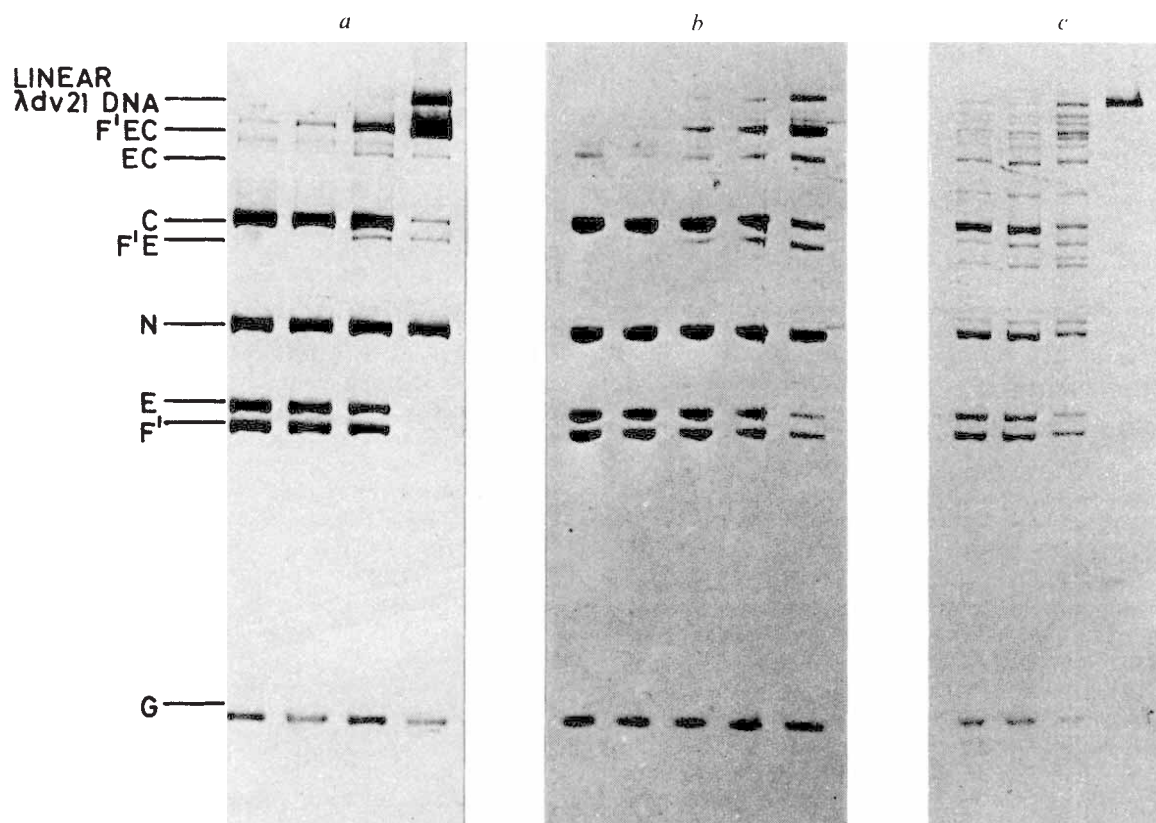


Fig. 2 Polyacrylamide gel electrophoresis of linear λ dv21 DNA cleaved with *Hind*II after complex formation with increasing amounts of histones. *a*, H2A (10%, 20%, 40% and 100% by weight of λ dv21 DNA); *b*, $(H3)_2(H4)_2$ tetramer (0%, 5%, 20%, 40% and 100%); *c*, equimolar mixture of H2A, H2B, H3 and H4 (10%, 20%, 40% and 100%). λ dv21 plasmid DNA was isolated and converted to the linear form with restriction nuclease *Hind*III as described¹¹. DNA concentrations were determined spectrophotometrically ($1 \text{ mg ml}^{-1} = 20 A_{260}$). Calf thymus histones were fractionated as described in ref. 14, except that 1 mM phenylmethyl sulphonyl fluoride (PMSF) was present throughout purification. The tetramer complex $(H3)_2(H4)_2$ (ref. 12) was purified as described¹³. No cross contamination of individual histones was detected on sodium dodecyl sulphate (SDS)¹⁵ or acid-urea gels¹⁶ (3–5% would have been seen), except for H2B which contained about 5% of H4. Concentrations of histones were measured spectrophotometrically using the extinction coefficients given in ref. 17 (for H2A, H2B, H3 and H4) and in ref. 18 (for H1). Polylysine and polyarginine (Sigma) had average molecular weights of 30,000 and 58,000, respectively. DNA (50 μ g) and histones (0–50 μ g) were mixed in small dialysis bags in a total of 0.35 ml and taken through a reconstitution procedure similar to that described in ref. 7. The following dialysis steps were carried out: 5 M urea, 3 M NaCl, buffer (8 h); 5 M urea, 0.6 M NaCl, buffer (16 h); 0.6 M NaCl, buffer (8 h); buffer (16 h). The buffer used was 10 mM Tris-HCl, pH 8.0, 1 mM mercaptoethanol, 1 mM PMSF. (PMSF did not inhibit the nucleases.) The solution of the 100% complexes (except for the H2A–, tetramer–, and the nucleosome–complexes) and of the 40% complexes of H1, polylysine, and polyarginine were turbid; in all other cases clear solutions were obtained. The recovery of complexed DNA was about 80%. Equal amounts of the complexes were digested for 1 h at 37 °C with just sufficient restriction nuclease *Hind*II for complete cleavage of the free DNA. After addition of SDS and NaClO₄ (final concentrations 1% and 1 M, respectively) the histones were extracted three times with 2 volumes of phenol-chloroform-isoamyl alcohol (24:24:1); the DNA fragments were then precipitated with 2.5 volumes ethanol at –20 °C overnight, and analysed on 4% polyacrylamide slab gels essentially as described¹⁹. The smallest fragment F'' is not shown. The extracted histones were dialysed against 1 mM PMSF, lyophilised and analysed on SDS gels¹⁵ for proteolysis during the reconstitution and digestion procedures. No degradation was observed. The letters used for the indicated fragments are those of Fig. 1.

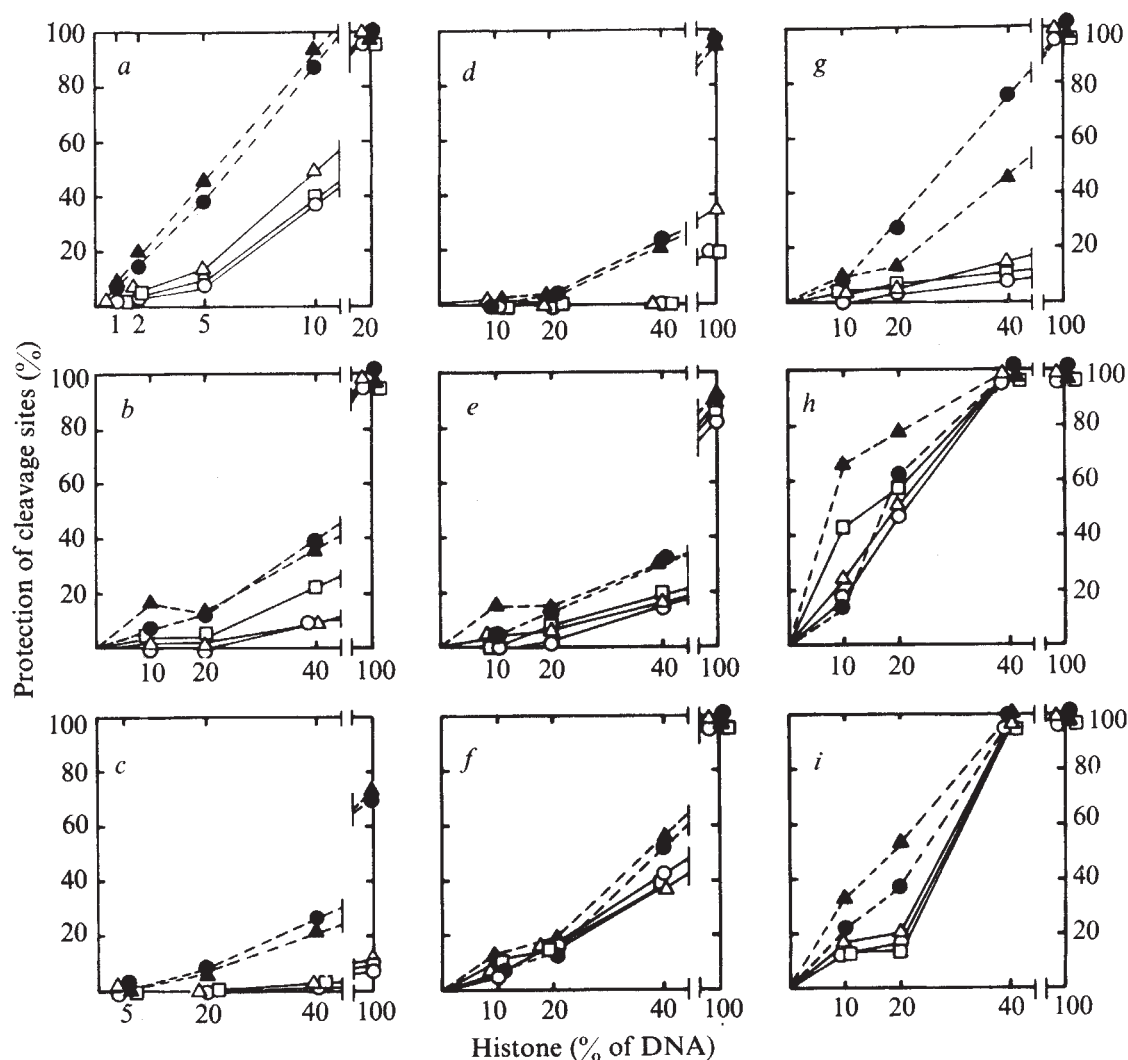


Fig. 3 Quantitative analysis of protection of *Hind*II cleavage sites in linear λ dv21 DNA by histones and polylysine. Polyacrylamide gel electrophoretic separations of DNA fragments of the *Hind*II-cleaved complexes were stained (Fig. 2), scanned and the peak areas of the densitograms (Fig. 1b) were measured planimetrically¹⁹. The extent of protection of a particular cleavage site was calculated from the relative amounts of the fragments in the digest. From the molecular weights of the fragments their molar amounts were obtained. Based on these data, the extent (on a molar basis) to which each of the cleavage sites was protected was calculated. Symbols used for the cleavage sites are those of Fig. 1a. a, H1; b, H3; c, (H3)₂(H4)₂; d, H2A; e, H4; f, H2A+H2B+H3+H4; g, H2B; h, polylysine; i, H1+H2A+H2B+H3+H4.

of urea was digested with micrococcal nuclease. No discrete DNA fragments were found when complexes of DNA with the histone pairs H2A+H2B or H3+H4 were digested. This agrees with the observation that all four histones are required to reconstruct the subunit structure^{5,22}. It should be mentioned that in the complexes prepared in the absence of urea, the preferential protection of the two left-hand *Hind*II cleavage sites by individual histones (H1, H2A, and the tetramer (H3)₂(H4)₂ were tested) was maintained and the unspecific protection by nucleosomes was unchanged.

The individual histones seem to be located mainly at or near the preferentially protected cleavage sites. It is also conceivable that they are randomly distributed but bind in some way specifically to those sites. The absence of specificity of protection by nucleosomes indicates that they are distributed randomly along the DNA. We conclude that interactions between the four histones leading to nucleosome formation change the binding behaviour of the histones to DNA in a pronounced way. H1 when present in addition to the nucleosome-forming histones may interact separately with the DNA, either between the nucleosomes or on the outside of the nucleosomes, thus giving rise to a specificity

of protection superimposed on the unspecific protection by the nucleosomes.

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Restriction nucleases as probes of chromatin structure

NUCLEASE digestion experiments have contributed greatly to our understanding of the structure of chromatin¹⁻⁴. Stretches of DNA protected by histones against nuclease attack seem to alternate with regions which are more easily accessible to nucleases. Together with electron microscopic studies, these findings have led to the proposal of the nucleosome model for chromatin⁵⁻⁷. Only nucleases cleaving DNA rather nonspecifically have been used so far in these investigations. It seemed promising at this stage to use restriction nucleases^{8,9} which have become most valuable in studies on the organisation of eukaryotic DNA.

We have found that the restriction nucleases *Bsu* and *EcoRII* can enter rat and mouse liver nuclei and overcome the protection of the DNA by the nuclear proteins. The average molecular weight of the DNA could be continuously shifted from more than 30×10^6 obtained in the absence of nuclease down to 2×10^6 for the highest nuclease concentration tested. It was surprising to see in the same digests an appreciable proportion of fragments in a regular pattern, similar to the one observed with the endogenous nuclease or with small amounts of micrococcal nuclease. The satellite DNA from mouse was shown to be accessible to *EcoRII* although most of it is contained in the highly condensed heterochromatin¹⁰.

Figure 1 shows how the molecular weight of the DNA from rat liver nuclei decreases after incubations with increasing amounts of *Bsu*. The presence of endogenous

nucleases, some of which are activated by Mg^{2+} , had complicated our early digestion experiments with restriction nucleases. In the incubation conditions now used, however, the DNA from nuclei incubated in the absence of *Bsu* for 3 h was only slightly smaller than that extracted from non-incubated nuclei. Very high restriction nuclease concentrations were required to split DNA in nuclei. The *Bsu* concentration which yielded a molecular weight distribution of the DNA around 2×10^6 was 80 times that required to completely digest free DNA in the same conditions. Mouse liver nuclei gave analogous DNA patterns after incubation with *Bsu*. The DNA molecular weight distribution shown for the highest *Bsu* concentration probably does not represent a limit digest. Extremely high *Bsu* concentrations will be required to completely overcome the protection of the DNA by the nuclear proteins. Intermediate concentrations will clearly be preferable to prepare chromatin with high molecular weight DNA.

When *Bsu* digests of mouse and rat liver nuclei were analysed on 1.5% agarose gels, a different picture emerged. Several per cent of the material followed a pattern similar to the one found with small amounts of micrococcal nuclease (Fig. 2). These patterns contain a series of bands which are integral multiples of a smallest fragment 180-200 nucleotide pairs long^{2-4,6}. The *Bsu* generated patterns characteristically contain mostly DNA fragments of multiple unit length with little 'monomer' and 'dimer' present.

Small amounts of bands in the 200 nucleotide pair register were also obtained from mouse liver nuclei with *EcoRII* (W.H., unpublished). From control experiments (Fig. 2a and b) we can rule out that these fragments are produced by endogenous nucleases. It is also highly unlikely that they are produced by nonspecific nuclease activities in the *Bsu* preparation, as λ dv1 plasmid DNA yields the usual 15 sharp bands¹⁴ in the conditions of chromatin digestion with the high *Bsu* concentrations. The bands also remain sharp when the λ dv1 DNA was added to chromatin and digestion carried out as described in Fig. 1. Additional sharp bands which can be produced from phage DNA with

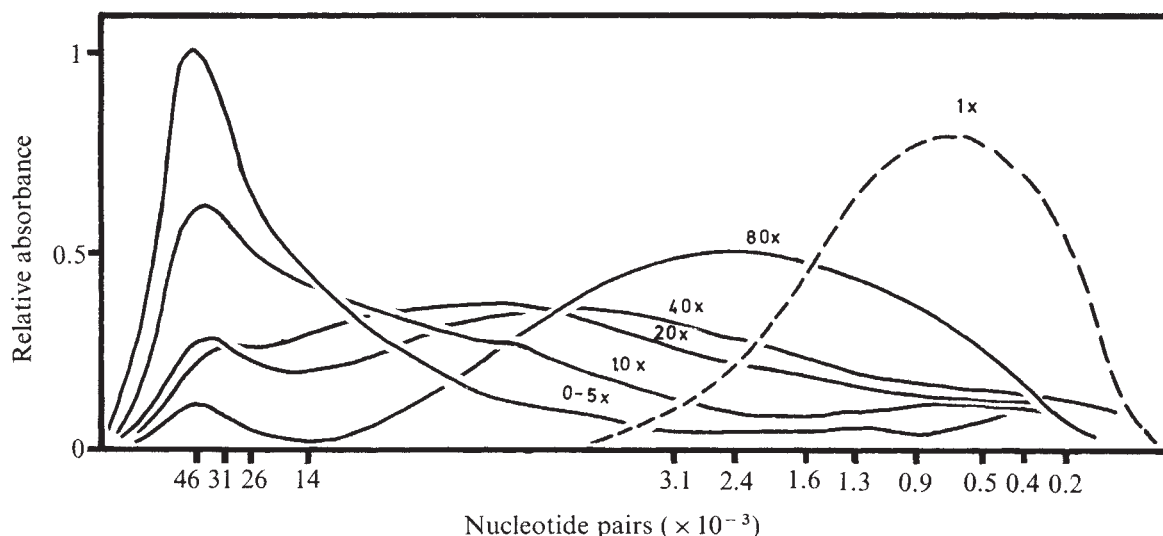


Fig. 1 Digestion of DNA in rat liver nuclei with *Bsu* analysed on 0.3% agarose gels. Nuclei were prepared as described previously¹ and used directly or frozen as a pellet in liquid nitrogen and resuspended by gentle homogenisation before use. Aliquots containing 0.5 A_{260} units DNA were incubated in 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 0.2 mM EDTA, 0.2 mM EGTA, 15 mM β -mercaptoethanol, 15 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$ with different *Bsu* concentrations. Incubations were for 3 h at 37 °C in a total volume of 50 μ l. *Bsu* was prepared as described previously¹¹. The incubations were terminated by the addition of EDTA, sodium perchlorate, and SDS to give final concentrations of 50 mM, 1 M and 1%, respectively; DNA was extracted twice with phenol, once with chloroform-isoamylalcohol (24:1, v/v), precipitated with 2 volumes ethanol, and dissolved in 10 mM Tris-HCl, pH 7.4, 1 mM EDTA. 0.05 A_{260} units of DNA were applied to a 0.3% horizontal agarose gel, run in the Tris-borate buffer system of ¹² including 1 μ g ml⁻¹ ethidium bromide. Time of electrophoresis was 36 h at 20 V. T5 DNA digested with *EcoRII*¹³ and λ dv1 plasmid DNA digested with *Bsu*¹⁴ served as molecular weight markers. At the end of the run the gel was photographed in ultraviolet light (Osram HQV lamps) with a Durst Laborator 138S equipped with an orange filter (Schott OG570). A graphical film (Agfa P23p, 13 $\times$ 18 cm) was used. The negatives were scanned and the peak areas normalised. Peak areas were shown to be directly proportional to the amounts of DNA over at least a factor of 10. The numbers refer to the concentrations of *Bsu* used. 1 $\times$ *Bsu* is the concentration required to completely degrade free DNA in 3 h in these conditions. - - -, Complete digest of free DNA with *Bsu*; —, DNA from rat liver nuclei digested with increasing concentrations of *Bsu*.

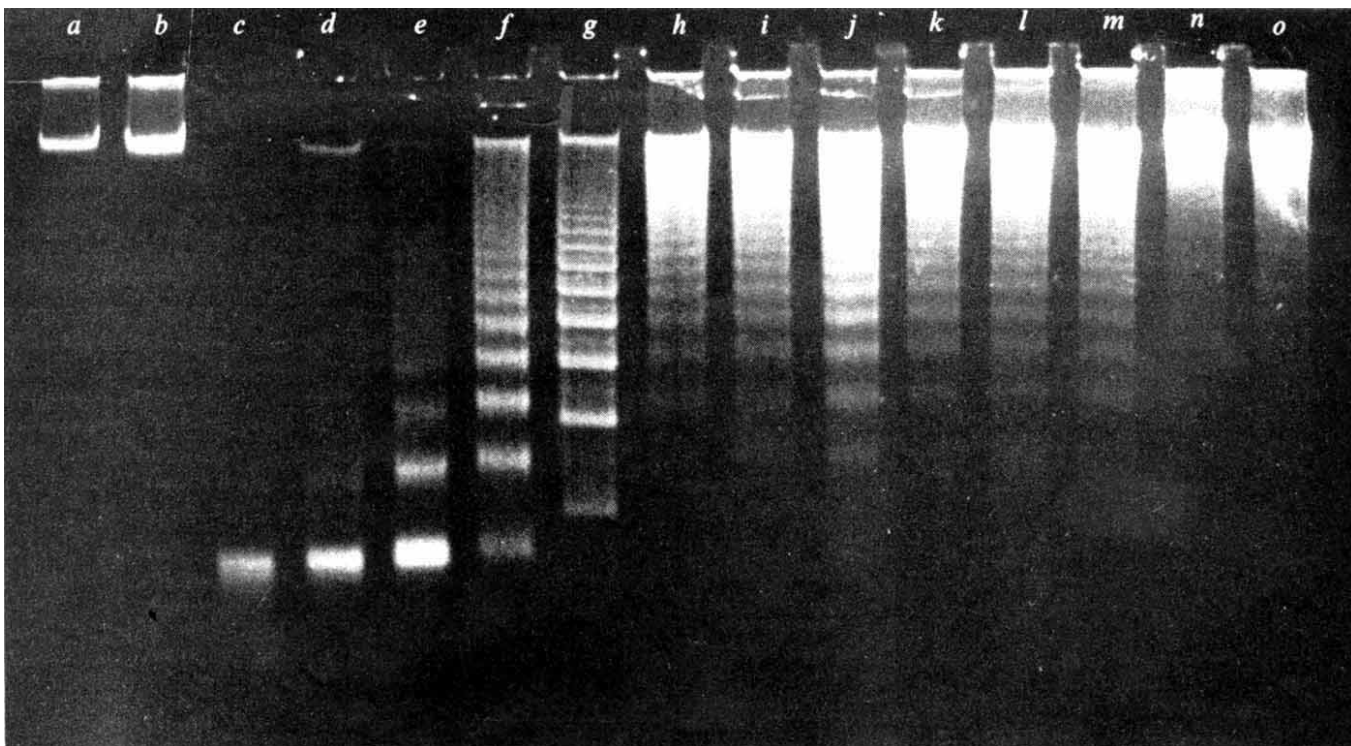


Fig. 2 Digestion of DNA in mouse liver nuclei with micrococcal nuclease and *Bsu* analysed on 1.5% agarose gels. Mouse liver nuclei were incubated in the absence or presence of nuclease for different periods of time, and the DNA extracted and analysed as described in Fig. 1 except that a 1.5% vertical agarose slab gel was used. When micrococcal nuclease was used, MgCl_2 in the incubation medium was replaced by 1 mM CaCl_2 . DNA samples from nuclei digested with micrococcal nuclease (Worthington) are in slots *c-f* (1 h; 30, 10, 3, 1 U ml^{-1}) and with *Bsu* in slots *h-o*. *h-j*, $100 \times Bsu$ (1 h, 2 h and 3 h); *k-m*, $40 \times Bsu$ (1 h, 2 h, and 3 h); *n-o*, $20 \times Bsu$ (1 h and 3 h). $1 \times Bsu$ was the concentration required to completely digest free mouse DNA in 3 h in identical conditions. DNAs in slots *a* and *b* are from control incubations of nuclei (1 h and 3 h) in the *Bsu* conditions. Slot *g* contains a mouse satellite DNA marker partially digested with *EcoRII*¹⁵. Analogous patterns were obtained from rat liver nuclei incubated with *Bsu* and micrococcal nuclease.

Bsu in different conditions (W.H. and K. Heininger, unpublished) were absent in both the control experiments. We cannot exclude the participation of endogenous nucleases but this only after a primary action of the restriction nuclease. The quantity of DNA falling into the regular pattern is definitely higher than one would expect on a statistical basis. The material in the 600 nucleotide pair region, for example, constitutes 1–4% of the total digest. If *Bsu* targets were randomly distributed in accessible segments of the nucleosomes of no more than 20 nucleotide pairs length one would expect less than 0.3% of the DNA in the 600 nucleotide pair region. Possible reasons for the higher experimental values could be a non-random distribution of *Bsu* sites or some basic difference between DNA and chromatin in restriction nuclease digestion.

The experiments aiming at the satellite DNA component of the nuclei were based on the finding that mouse satellite DNA is cleaved into a series of characteristic fragments by *EcoRII*¹⁵. When mouse liver nuclei were digested with *EcoRII* one could indeed detect these fragments at a high enzyme concentration (Fig. 3). This shows that a sizeable proportion of cleavage sites must have been available for the nuclease, otherwise much larger fragments would have been produced instead. It will be interesting to determine in detail how the degree of accessibility of satellite DNA in nuclei compares with that of the other

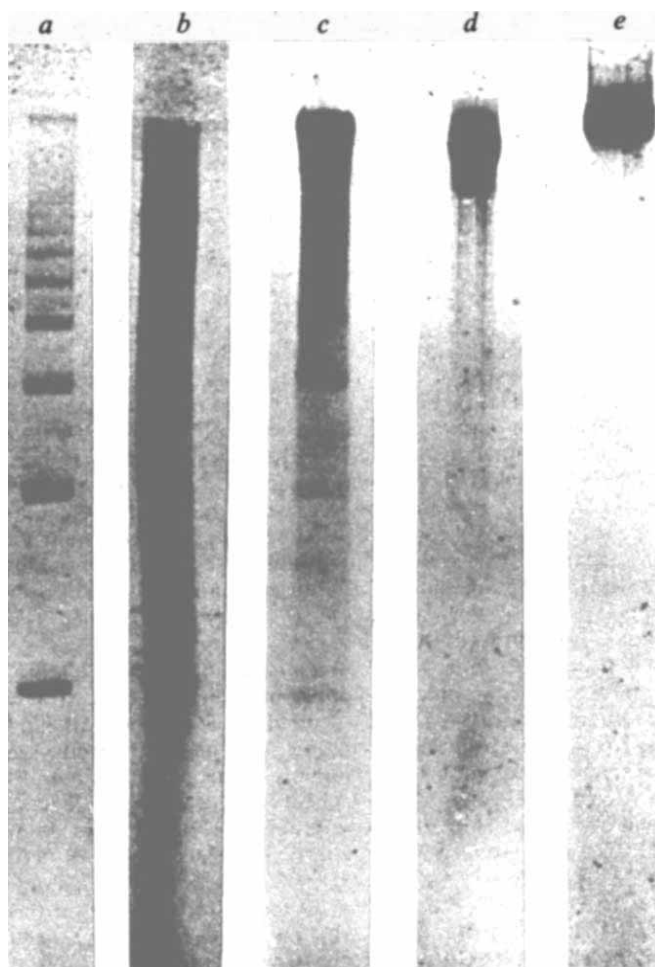


Fig. 3 Digestion of mouse DNA and nuclei with *EcoRII*. 0.05 A_{260} units of mouse satellite DNA (slot *a*) and 0.2 A_{260} units unfractionated mouse DNA (slot *b*) were incubated with $0.3 \times$ and $1 \times EcoRII$, respectively, for 3 h and the digests analysed on 4% polyacrylamide gels as described previously¹⁶. *EcoRII* was prepared according to ref. 17. $1 \times EcoRII$ is the concentration required to completely digest free DNA in 3 h. In slots *c* and *d*, DNA from mouse nuclei incubated for 3 h with $20 \times EcoRII$ and $4 \times EcoRII$ in the conditions of Fig. 1 was analysed. Slot *e* contains DNA from control nuclei incubated for 3 h in the absence of nuclease.

DNA and further if the repetitive DNA also conforms to the nucleosome substructure of chromatin.

Our results show that digestion of chromatin with restriction nucleases yields information not available with other nucleases. Note also the possibility of using the more defined digestion of whole nuclei by restriction nucleases for preparative purposes, thus circumventing the random shearing methods used previously. This approach should complement and extend the recently introduced digestion of nuclei with nonspecific nucleases for chromatin preparation¹⁸.

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Changes in form of elongation factor during development of *Artemia salina*

It is well established that elongation factor 1 (EF1), the enzyme responsible for binding aminoacyl tRNA to ribosomes, exists in multiple forms in a variety of different eukaryotic tissues¹⁻⁶. It is also generally accepted that in mammalian cells the heavy forms of EF1 (EF1_H), with molecular weights ranging from 2×10^5 to $>1 \times 10^6$, represent aggregates of a light form, with a molecular weight of $\sim 50,000$ (refs 4 and 7). The functional significance of these findings remains unclear, since both forms are active in binding aminoacyl tRNA to ribosomes. We report here that the dehydrated gastrulae (cysts) of the brine shrimp, *Artemia salina*, contain almost exclusively a high molecular weight ($\sim 250,000$) form of EF1. During development of the organism EF1_H seems to disappear completely so that the free swimming (hatched) embryos (nauplii) contain only a light form of the enzyme (EF1_L). This change occurs at or near the time of hatching.

We purified EF1 from *Artemia* cysts to near homogeneity using ammonium sulphate precipitation followed by chromatography on DEAE-Sephadex, Sepharose 6B and hydroxylapatite (see legend to Fig. 1). The purified enzyme gave one band on gel electrophoresis at pH 8.9 and sedimented at the same position as beef liver catalase (molecular weight 250,000) on a sucrose gradient (Fig. 1). Based on SDS gel electrophoresis, the enzyme consists mainly of subunits of molecular weight 25,000-30,000, although a component of 60,000 was also observed. Similar observations on the subunit structure of wheat embryo EF1_H have been made by Bollini *et al.*⁸. The characterisation of the subunit

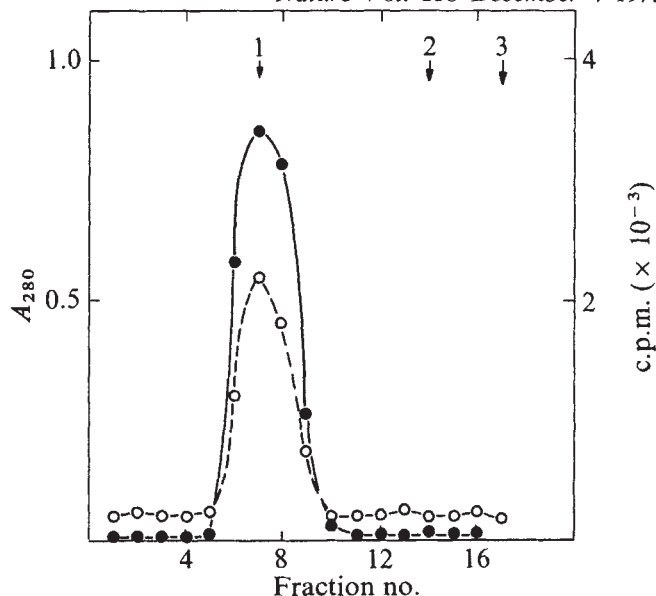


Fig. 1 Sedimentation on a sucrose gradient of purified EF1 from *Artemia* cysts. EF1 was purified as follows: all operations were performed at 2-4 °C. Cysts were washed with NaOCl and water, and ground in a mortar at 0 °C as described¹³. Nauplii were washed extensively with 4% NaCl before preparation of extracts. Both ground cysts and nauplii were suspended in extraction buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM mercaptoethanol, 5 mM magnesium acetate, 5% glycerol (w/v)) and the post-ribosomal supernatant obtained by a series of centrifugations at 2 °C: 5 min at 2000g, 10 min at 33,000g and finally 90 min at 50,000 r.p.m. (150,000g) in the Beckman 60 Ti rotor to pellet the ribosomes. The supernatant from each centrifugation was poured through a glass wool plug. Care was taken to avoid the upper lipid layer after the last centrifugation. The protein fraction of the post-ribosomal supernatant which precipitated between 30 and 70% saturated ammonium sulphate was dialysed against a buffer containing 20 mM Tris-HCl, pH 7.5, 150 mM KCl, 10 mM 12-mercaptoethanol, 0.1 mM EDTA and 1 mM magnesium acetate and applied to a column of DEAE-Sephadex G-50 previously equilibrated with the same buffer. EF1 was not retained by DEAE-Sephadex and was collected in the eluate, concentrated by ammonium sulphate precipitation, and chromatographed on Sepharose 6B, using the same buffer as above except that the KCl concentration was 100 mM. The fractions containing EF1 activity were concentrated and, after dialysis against 50 mM KPO₄ buffer, pH 7.5, containing 10 mM 2-mercaptoethanol and 10% glycerol, applied to a column of hydroxylapatite (BioRad HTP). The column was developed with a linear gradient between 50 mM KPO₄ and 250 mM potassium phosphate buffer, pH 7.5, containing 10 mM 2-mercaptoethanol and 10% glycerol. EF1 eluted at 200 mM phosphate. The active fractions were concentrated by ultrafiltration and rechromatographed on Sephadex G-200. One protein peak was obtained which had a specific activity (one unit of activity is defined as the amount of protein required to bind one 1 pmol of Phe tRNA in the standard assay (see below), ranging from 750-1,000 for different EF1 preparations. Purified EF1 (100 µl of a 1% solution) was applied to 5 ml of a 10-30% linear sucrose gradient in a buffer containing 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM mercaptoethanol and 1 mM magnesium acetate. The sample was centrifuged for 18.5 h at 2 °C and 40,000 r.p.m. in a Beckman/Spinco SW 50.1 rotor. After centrifugation, the tube was pierced at the bottom and 16-drop (~ 0.3 ml) fractions were collected. Aliquots (25 µl) of each fraction were assayed for EF1 activity. Assay mixtures (100 µl) contained the following components: 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM mercaptoethanol, 5 mM Mg(OAc)₂, 1.0 A₂₈₀ units of 0.5 M KCl washed 80S ribosomes from *Artemia* cysts⁹, 0.25 mM GTP, 20 µg of poly(U) and approximately 35 µg of *Escherichia coli* tRNA charged with 13 pmol ($\sim 10,000$ c.p.m.) of ³H-phenylalanine (1.1 Ci mmol⁻¹). After incubation for 20 min at 30 °C, the reaction mixtures were diluted with ice-cold buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl and 5.0 mM magnesium acetate) and collected by suction on to 0.45-µm nitrocellulose filters (Sartorius). The filters were washed 3 times with 3-ml portions of cold buffer, dried and counted with 30% efficiency in a Packard Tricarb Liquid Scintillation Spectrometer. Marker proteins, designated by the arrows, in a volume of 100 µl were run on a separate gradient and their absorbance measured. 1, Bovine catalase (S_{20,w} = 11.7, molecular weight 247,000); 2, bovine serum albumin (4.3, 66,000); 3, horse heart cytochrome c (1.7, 13,400). —, A₂₈₀; - - - -, c.p.m.

structure of the enzyme is currently under investigation. Our observations regarding the size of the native enzyme in *Artemia* cysts are in agreement with those of Nombela and Ochoa⁹.

To establish whether EF1_L is also present in *Artemia* cysts the post-ribosomal supernatant (PRS) of extracts of *Artemia* cysts was analysed for EF1 activity after centrifugation on a sucrose gradient. The results (Fig. 2a) show that most of the EF1 activity sediments as high molecular weight forms ($S>5$), with the bulk sedimenting in a range roughly comparable with that of the purified enzyme.

To determine the form of EF1 which is present in nauplii, *A. salina* embryos were developed for 45 h at 25 °C in 4% artificial seawater. In these conditions >90% of the embryos hatched to form free-swimming nauplii. When the PRS of extracts of nauplii were examined on sucrose gradients, there was a remarkable change in the EF1 pattern (Fig. 2b). All EF1_H had disappeared and was replaced by an activity which cosedimented with bovine serum albumin ($S_{20,w}=4.3$), indicating the presence in nauplii of EF1_L exclusively. We next investigated the time during development at which the conversion to EF1_L occurs. *Artemia* cysts were developed as before for 26 h; approximately 40% of the embryos were present as nauplii, as judged by microscopic analysis. Examination of the PRS after 26 h of development on sucrose gradients showed that approximately 40% of the EF1 activity was in the form of EF1_L. The remaining activity was present as higher molecular weight forms. Since about 40% of the organisms

Fig. 2 Sucrose gradient patterns of EF1 activity of cysts and nauplii of *A. salina*. PRS was prepared as described in legend of Fig. 1. Freshly prepared PRS (100 μ l) was applied to 5 ml 10–30% sucrose gradients. The gradients were analysed for EF1 activity as described in Fig. 1 using 20- μ l aliquots from each fraction (16 drops). In the assay conditions EF1 was limiting. *a*, PRS from cysts (4.4 mg protein per ml) was centrifuged for 16 h at 42,000 r.p.m. in the SW 50.1 rotor; *b*, PRS from nauplii (10 mg protein per ml) was centrifuged for 16 h at 42,000 r.p.m.

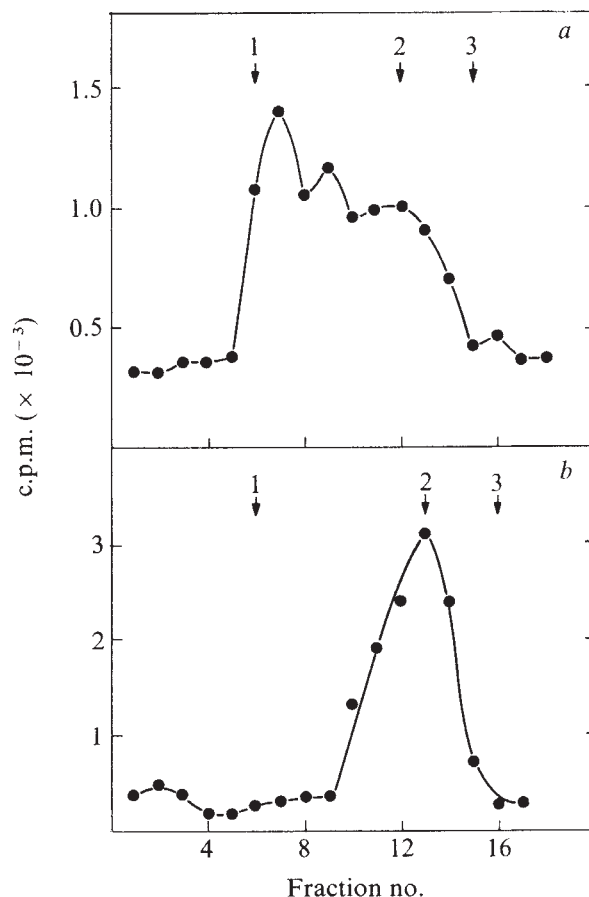
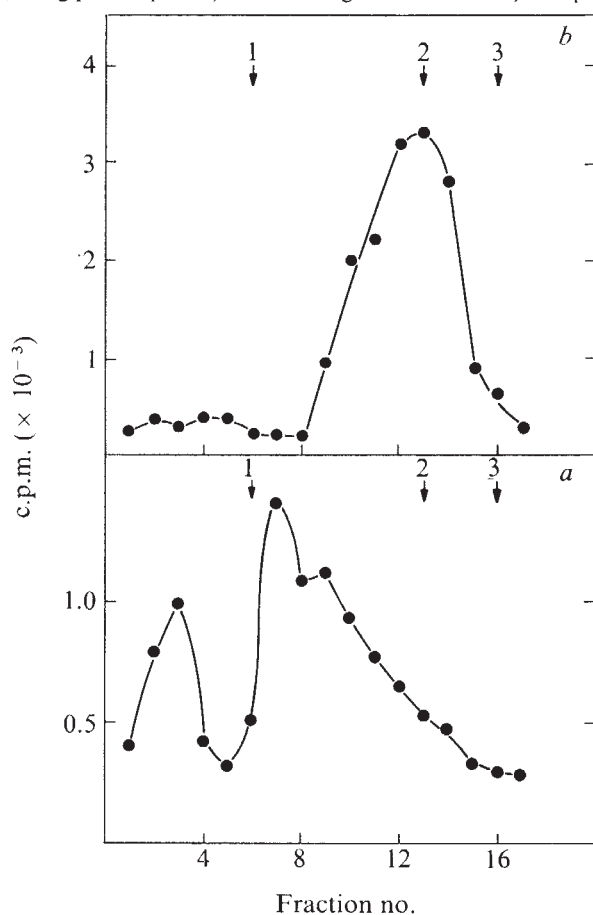


Fig. 3 Sucrose gradient patterns of EF1 activity after 26 h of development. Extracts were prepared and analysed as described in the legend to Fig. 2. *a*, PRS (100 μ l) of *A. salina* after 26 h development, consisting of a mixture of cysts and nauplii (see text), containing 7.2 mg protein per ml was centrifuged at 42,000 r.p.m. for 15 h; *b*, PRS (100 μ l) of isolated *A. salina* nauplii after 26 h development (see text), containing 8.2 mg protein per ml was centrifuged at 42,000 r.p.m. for 16 h.

were free swimming, the distribution of activity could reflect an average value, in which EF1_L exists exclusively in nauplii, whereas EF1_H is present in unhatched cysts. Alternatively, newly emerged nauplii may have the same distribution of the different forms of EF1 as pre-emergent cysts. The exclusive presence of EF1_L after 48 h of growth could then be explained by a gradual conversion of EF1_H to EF1_L during development.

To investigate these two possibilities, cysts were again developed for 26 h, at which time nauplii were separated from developing cysts and pre-emergent nauplii. This separation was accomplished essentially as previously described¹⁴. The EF1 pattern of nauplii purified thus is shown in Fig. 3b; only EF1_L is present in these newly hatched embryos. Consistent with these findings we have observed no significant change in the EF1 patterns during the early development of the cyst (6 h at 25 °C); only after 16 h, when approximately 10% hatching has occurred, is a peak of EF1_L activity clearly visible (unpublished).

We have attempted to convert EF1_H to EF1_L *in vitro*. Purified EF1_H was incubated with varying amounts of PRS from both cysts and nauplii. In preliminary experiments neither supernatant was found to have any effect on the sedimentation behaviour of EF1_H. Lanzani *et al.*¹⁰ have reported that cyclic GMP caused a small conversion of EF1_H to a lighter form of the enzyme. In these studies cyclic GMP also seemed to stimulate the binding of GTP by EF1_H as well as factor-dependent polyphenylalanine synthesis. To test the possibility that cyclic nucleotides

Table 1 Effect of cyclic GMP on EF1 activity

Cyclic GMP (mM)	Phe tRNA bound c.p.m. ($\times 10^{-3}$)	GTP bound c.p.m. ($\times 10^{-3}$)
0	1.9	8.6
0.01	1.6	—
0.02	—	7.4
0.04	—	6.3
0.1	1.9	—

The binding of the Phe tRNA to ribosomes was determined as described in the legend to Fig. 1 except that 3 μ g of purified EF1_H was used and the incubation time was reduced to 10 min. In these conditions the amount bound to ribosomes was found to directly proportional to EF1 concentration. The binding of GTP (0.01 mM final concentration, specific activity 2 Ci mmol⁻¹) to EF1 was determined by a nitrocellulose filter assay as described⁹. Each assay mixture contained 20 μ g of purified EF1_H.

may regulate the conversion of EF1_H to EF1_L, purified EF1_H from *Artemia* cysts was assayed in the presence of cyclic GMP. It may be seen (Table 1) that cyclic GMP has no effect on the activity of *Artemia* EF1_H to bind aminoacyl tRNA to ribosomes, but causes a slight inhibition of the GTP binding capacity of the factor. In addition, no significant conversion of EF1_H to EF1_L was observed in the presence of cyclic GMP. We conclude that cyclic GMP is not directly responsible for the conversion of *Artemia* EF1_H to EF1_L.

The dormant cysts of *A. salina* consist of approximately 4,000 cells (see ref. 11 for a review) which contain a large store of unprogrammed ribosomes¹² as well as EF1, EF2 (ref. 9) and EIF1 (ref. 13). On exposure of the cyst to the right conditions, protein synthesis is gradually resumed and extensive morphogenesis occurs in the absence of cell division¹¹. After hatching, a burst of RNA and DNA synthesis occurs followed by cell division¹⁴. The conversion of EF1_H to EF1_L seems to coincide with these later events. Although we have not shown a direct precursor-product relationship between EF1_H and the EF1_L of nauplii, it seems likely that such a relationship, in which EF1_H represents a storage form of the enzyme, exists. The mechanism by which EF1_H is converted to EF1_L is at present under study.

The coincidence between the conversion of EF1_H to EF1_L and the rapid synthesis of nucleic acid also raises the possibility that EF1, as is the case with its bacterial counterpart TuTs¹⁵, has a function in metabolism other than in protein biosynthesis. Certainly the observation, that the form of EF1 present in all cells of a multicellular organism is correlated with a morphogenetic event, suggests that the occurrence of these forms has real physiological significance. This work was supported by a grant from the US National Science Foundation.

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Candidate leukaemia-specific antigen in man

It is well established that distinct changes in the antigenic phenotype of the cell surface accompany viral transformation. The 'new' determinants may be viral, oncofoetal (or 'phase' specific) or tumour specific¹⁻³. The identification of neoantigens, and particularly putative tumour specific antigens, on human neoplasms opens up possibilities of early and precise diagnosis, patient monitoring and immunotherapy. It may also eventually provide information relevant to the aetiology of the disease. We report here the demonstration of a cell surface antigen present on leukaemic cells from patients with acute lymphoblastic leukaemia (ALL).

Studies with cell surface differentiation markers for distinctive lymphocyte populations have been useful in analysing the phenotype and possible origin of human leukaemic cells (reviewed in refs 4 and 5). ALL seems to be a heterogeneous disease which may, nevertheless, be divisible into two major categories. Approximately 25% of ALL cases have a distinctive T-cell surface phenotype often associated with a mediastinal (thymic) mass or enlargement. The remainder and majority (75%) of the cases seem to lack reactivity with all available cell-surface markers for T and B lymphocytes. Relatively rare cases (1-2%) have a B-lymphocyte phenotype and are considered to be a 'Burkitt lymphoma type'⁶ on morphological grounds. It is therefore possible that most patients with ALL have a neoplastic disease which originates in a relatively undifferentiated cell which is either committed to the lymphoid developmental pathway or is a stem cell with wider potentialities.

Several laboratories have reported encouraging results with heteroantisera to leukaemia-associated antigens⁷⁻¹¹. We have raised antisera to leukaemic blasts from the major class of ALL (that is 'non-T, non-B') and have used these sera to define leukaemia-associated or leukaemia-specific cell surface determinants. Detailed characteristics of these sera and their diagnostic applications have been published^{5,11,12}.

Antisera were raised in rabbits and absorbed as described in Table 1 (see also ref. 11). Binding was assessed by indirect fluorescence (with a goat anti-rabbit Ig conjugate) using both a Zeiss photomicroscope with Plume incident illumination and the Fluorescence Activated Cell Sorter, FACS-1 (Becton Dickinson)¹³. After initial absorptions with red cells, liver and tonsil lymphocytes, the antisera seemed to be ALL specific; however, careful analysis using the FACS-1 revealed that reactivity remained against a small population of mononuclear cells in non-leukaemic bone marrow samples. These reactive cells were purified using FACS-1 and were found to be myelocytes (which reacted very weakly) and a subset of intermediate

Table 1 Reactivity of anti-ALL sera with various human leukaemias

	% Positive cases						
	ALL	Other leukaemias					
Non-T Non-B	T	B	AML	AMML	AUL	CLL	CML
47/50	0/13	0/3	1/48	0/10	6/8	0/6	0/5

All untreated patients, blood and bone marrow samples. Anti-ALL sera were raised by injecting 2×10^8 viable ALL (non-T non-B variety) cells intravenously into rabbits. Before injection, cells were incubated for 2 h with rabbit antibodies produced against normal lymphocyte antigens (see ref. 11 for further details).

Leukaemia type was diagnosed by conventional morphological and histochemical criteria (for example, Sudan black, periodic and Schiff staining). ALL, acute lymphoblastic leukaemia (children and adults); AML, acute myeloid leukaemia; AMML, acute myelo-monocytic leukaemia; AUL, acute undifferentiated leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; T, (thymus derived) cell-like membrane phenotype (that is E rosette positive, anti-T serum positive¹⁹); B, ('bursa' equivalent derived) cell-like membrane phenotype (that is anti-immunoglobulin positive)¹⁹.

Table 2 Possible antigenic specificities defined by antisera to ALL

Potential antigen	Cells tested	Cross reactive with anti-ALL*
'Normal' antigen	Thymus†, tonsil†, appendix, blood WBC, spleen, bone marrow, liver	No‡
'Cryptic' normal lymphocyte antigen	Tonsil lymphocytes treated with trypsin§, Pronase or neuraminidase¶	No
Lymphocyte antigens expressed during mitosis	Mitogen activated tonsil T and B lymphocytes** Lymphoblastoid cell lines†† Lymphoblasts from blood of patients with infectious mononucleosis	No
'Foetal' antigens	Spleen 13–22 weeks Thymus 15–22 weeks Liver (9–22 weeks)†	No

* All cells were tested by indirect immunofluorescence with anti-ALL.

† Cells also tested for their capacity to absorb out anti-ALL antibodies.

‡ No reactivity = less than 0.5% positive cells recorded which is the 'background' with normal control sera, or alternatively, in absorption experiments, reflects no shift in the FACS profile on anti-ALL against ALL cells.

§ Trypsin (Sigma 12,000 BAEE U mg⁻¹, twice crystallised) 50 µg per 10⁶ cells per ml per 30 min per 37 °C.

¶ Pronase (BDH 45,000 PuK units per g) 50 µg per 10⁶ cells per ml per 30 min per 37 °C.

¶ Neuraminidase (*Vibrio cholera*, Calbiochem, grade B) 0.5 U per 10⁶ cells per ml per 30 min per 37 °C.

** Tonsil T lymphocytes cultured with phytohaemagglutinin or concanavalin A or spleen B lymphocytes cultured with pokeweed mitogen (see ref. 20 for method).

†† Derived from normal adult blood or from blood of patients with infectious mononucleosis, Burkitt's lymphoma or ALL (see ref. 11)

normoblasts (which reacted very strongly)¹². Most intermediate normoblasts in early (11–13 week) foetal liver were also strongly stained. The former cross reactivity could be removed by absorption with AML or bone marrow and both activities could be removed with either bone marrow or foetal liver. After this activity had been removed, staining of ALL cells remained and could not be significantly diminished by further absorption¹².

The reactivity of bone-marrow-absorbed anti-ALL sera with various leukaemias is given in Table 1. Virtually all non-T, non-B ALL cells react, whereas no T-cell ALLs or myeloid leukaemias stain. It is particularly interesting that six of eight cases of acute undifferentiated leukaemias (AUL) were positive (Table 1) as well as four of twelve cases of chronic granulocytic leukaemia (CGL) in blast crisis relapse (unpublished results of T. Revesz, G. Janossy, M.F.G., and M. Beard). These results suggest that these leukaemias share a common antigen which might reflect their origin from a common progenitor or leukaemia 'target' cell. ALL patients in remission have been studied and in a minority some staining cells were detectable¹². The possible significance of this result with respect to monitoring patients throughout treatment and predicting relapse has been discussed elsewhere^{5,12}.

The results suggested the existence of a leukaemia-specific antigen. We have therefore sought to eliminate unequivocally alternative interpretations. These are that the antigen is (1) foetal or oncofoetal; (2) a cell-cycle restricted determinant; (3) a normal cryptic determinant, or (4) a normal antigen present on a rare cell, for example, a stem cell. The first three of these were relatively straightforward to assess; the cell types tested are listed in Table 2. Within the sensitivity of our absorption and immunofluorescence tests, no evidence could be found to suggest or support a foetal, cryptic or cell-cycle association of the ALL antigen (Table 2). The fourth possibility is difficult to test. We can conclude that if the antigen is present on 'normal' cells in marrow then the frequency of reactive cells is certainly less than 0.5% and probably less than 0.1%. This level of detection is probably insufficient to exclude stem cells. This question has not been considered in previous analyses of

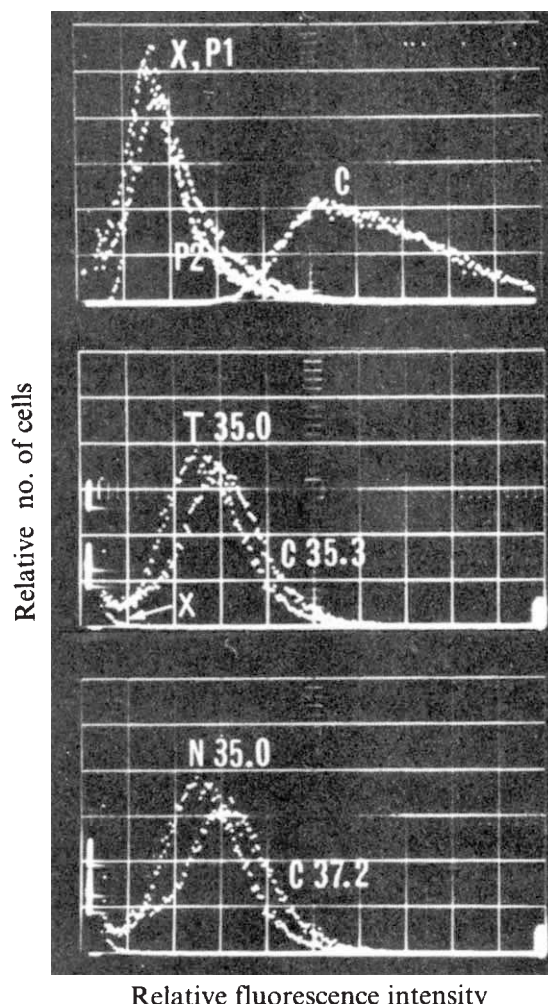


Fig. 1 Effect of Pronase, trypsin and neuraminidase on ALL associated antigens. FACS fluorescence profiles. A stock solution of Pronase (40 mg ml⁻¹ BDH 45,000 PuK units per g) was prepared in PBS. Two Pronase concentrations were used—1 mg per 10⁶ cells per ml and 10 mg per 10⁶ cells per ml. The cells were incubated with the enzyme for 1 h at 37 °C after which the enzyme was inactivated by the addition of 50 µl of foetal calf serum and incubating the cells at room temperature for 10 min. The cells were washed once with Earle's saline containing 10% foetal calf serum and tested for positivity with an anti-ALL serum, using the indirect immunofluorescence assay. FACS fluorescence profiles were compared for Pronase-treated and untreated cells or cells to which Pronase had been added and immediately inactivated. The viability of cell suspensions after treatment with Pronase, as assessed with Trypan blue, was > 99%. Similar experiments were carried out with trypsin. A 0.25% stock solution of trypsin (Sigma, 12,000 BAEE U mg⁻¹) was prepared in PBS. Time course and dose-response experiments were carried out using trypsin concentrations from 0.12 mg per 10⁶ cells per ml to 1.2 mg per 10⁶ cells per ml and incubation times from 0.5 to 1.5 h. The trypsin was inactivated by adding 50 µl of foetal calf serum and incubating the cells at room temperature for 10 min. The viability of all cell suspensions after trypsination and as assessed with Trypan blue was > 99%. A stock solution of neuraminidase (500 U ml⁻¹, *Vibrio cholera*, Calbiochem grade B) was prepared in PBS. Neuraminidase was used at concentrations of 0.5, 2.5, 5.0 and 10.0 U per 10⁶ cells per ml. Cells were resuspended in calcium saline and incubated with neuraminidase for 20 min at 37 °C. The viability of cell suspensions after treatment with neuraminidase was > 99%. C, ALL cells tested with anti-ALL serum; X, ALL cells tested with normal rabbit serum; P, ALL cells after Pronase treatment (P1, 1 mg per 10⁶ cells per ml; P2, 10 mg per 10⁶ cells per ml) and tested with anti-ALL serum. The fluorescence profiles in the above cases were moved to the right of the figure by increasing the laser power. This clearly demonstrates the similarity between P1, P2 and X. The following were recorded at the routine laser power: T, ALL cells after trypsination (1 h, 1.2 mg per 10⁶ cells per ml) and tested with anti-ALL serum; N, ALL cells after neuraminidase treatment 20 min, 10 U per 10⁶ cells per ml) and tested with anti-ALL serum. The numbers on the figures indicate the number of positive cells per 40,000 cells analysed.

Table 3 Co-capping of lectin binding sites and ALL antigen

Assay for uncapped sites		
Ligand	% Cells with ring fluorescence	
Lentil sites capped (64.8%)	Lentil	< 1
	Anti-ALL	< 1
Control	Lentil	100
	Anti-ALL	98
PHA sites capped (70.4%)	PHA	< 1
	Anti-ALL	> 99
Control	PHA	100
	Anti-ALL	98

Lens culinaris haemagglutinin (lentil) was purified from lentil using the method of Howard *et al.*²¹. Purified phytohaemagglutinin from *Phaseolus vulgaris* was obtained from Burroughs Wellcome. Rabbit antisera to PHA and lentil were prepared by two to four intramuscular or subcutaneous injections of 1 mg of lectin in Complete Freund's adjuvant. The co-capping experiments were carried out as follows. ALL blasts were treated with PHA (10 µg per 10⁶ cells per ml) or lentil (0.5 mg per 10⁶ cells per ml) for 30 min at 37 °C followed by appropriate saturation dilutions of rabbit anti-lectin sera (30 min, 37 °C) then fluorescein-labelled goat anti-rabbit antibody (30 min, 37 °C). Redistribution of lectin binding sites into discrete caps was observed and the number of capped cells was noted. These cells were then divided into two samples, one of which was tested in an indirect immunofluorescence assay at 4 °C in the presence of 0.2% sodium azide for reactivity with anti-ALL serum. The second was restained with lectin, rabbit anti-lectin and goat anti-rabbit antibodies, at 4 °C in the presence of 0.2% sodium azide. In both of these groups, cells pre-capped with lectin were assessed for additional ring staining which would indicate the presence of uncapped lectin binding sites or ALL antigen. Controls were untreated before reacting them with anti-ALL and lectin.

putative leukaemia-specific reagents. The lack of unequivocal proof of leukaemia specificity does not preclude the diagnostic application of the antiserum, and the results in this respect have already proved of considerable interest and value (Table 1 and refs 5 and 12).

As a prelude to isolation and purification of the ALL antigen we have investigated its general chemical nature. Treating ALL cells with enzymes (Fig. 1) revealed that the antigen was associated with a Pronase-sensitive but trypsin- and neuraminidase-insensitive structure.

To test whether the antigen was associated with a oligosaccharide-bearing molecule we investigated the capacity of lectins to redistribute the antigen within the lateral plane of the membrane. This 'co-capping' approach has proved valuable in revealing molecular interrelationships of cell-surface molecules¹⁴⁻¹⁶. The design of these experiments and the results observed are given in Table 3. It seems that lentil (*Lens culinaris*) lectin interacted (and co-capped) with the molecular entity on which the ALL antigen was expressed. In contrast, phytohaemagglutinin (*Phaseolus vulgaris*) binding sites and the ALL antigen seemed to be independent. We therefore suppose that the molecule of interest is probably a glycoprotein, although a strong non-covalent association with glycoprotein cannot be excluded.

The possibility that a virus is involved in human leukaemia is clearly of great potential importance. The most suggestive evidence so far comes from the isolation by Gallagher and Gallo of C-type particles from long term tissue culture of myeloid cells from an AML patient (HL23)¹⁷. A viral isolate from this patient, called HL23V-1, has been shown to be infectious for various cell types and to resemble closely the C-type simian sarcoma virus (SSV) isolated from a woolly monkey¹⁸.

We have therefore attempted to determine immunological cross reactivity between the ALL antigen described above, HL23V-1 and a number of other candidate human and animal leukaemia viruses. These experiments involved asking two related questions. First, can virus extracts absorb the anti-ALL activity? Second, do antisera to these same viruses react with ALL cells? The latter experiment is controlled by demonstrating reactivity with infected animal cells. These tests were carried out under quantitative conditions using FACS analysis. The

results showed that there is no immunological cross reactivity demonstrable by absorption between the ALL antigen and murine leukaemia virus (Gross and Moloney), feline leukaemia virus, HL23V-1, Newcastle disease virus and influenza virus (X31). Antisera to disrupted feline leukaemia virus (GaFeLV) which reacts with interspecies determinants on the P30 molecule and antisera to simian sarcoma virus P30 (RaSSVP30) or whole SSV showed no fluorescent labelling of ALL cells. Neither did antisera to Epstein-Barr virus, herpes saimiri, rubella and cytomegalovirus.

In spite of this impressive list of negatives we cannot and do not exclude viral involvement in ALL or in the expression of the ALL antigen. Clearly, however, these results diminish the possibility that the ALL cell-surface antigen we have identified is a virally coded determinant.

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Corrigendum

In the article "Differential effects of glucocorticoids on DNA synthesis in normal and virus transformed chick embryo cells" by D. W. Fodge and H. Rubin (*Nature*, **257**, 804; 1975) the right-hand axis of Fig. 3 is incorrectly labelled. It should read Labelled nuclei (fraction) and not (%) as printed.

Erratum

In the Matters Arising contribution "Sympathoadrenal medullary activity in young, spontaneously hypertensive rats" by H. Grobbeck, M. F. Roizen, V. Weise, J. M. Saavedra and I. J. Kopin (*Nature*, **258**, 267; 1975) the asterisked footnote to Table 2 should read ... *Results are expressed as µg of catecholamines, µg of noradrenaline and units of enzyme per adrenal gland and are the mean values (±s.e.m.) for groups of 14 animals ... and not as printed.

matters arising

On the 27-day cycle in the rainfall at Los Angeles

ROSENBERG and Coleman¹ reported a 27-d cycle in the rainfall of Los Angeles. Subsequently the former author has used the cycle to forecast, starting from an initial day to be determined each year, 13.5 d wet and 13.5 d dry periods in the winter months of Southern California. Their 27-d cycle was based on the spectral analysis of the rainfall data at the Los Angeles Civic Center. They used the Blackman-Tukey method with the Tukey spectral window² and a maximum of 250 lags. I question the significance of the peaks in the Los Angeles rainfall spectrum.

Professor M. Wurtele of the Department of Meteorology at UCLA provided a record of daily rainfall data for Los Angeles covering a period of over thirty years (June 1943–August 1973). (UCLA campus is ≈ 17 miles from the Los Angeles Civic Center.)

Spectral analysis of the UCLA rainfall data was done by the method of direct estimation of spectral density using Fast Fourier Transforms³. The detailed description of this method is found in Cooley *et al.*⁴.

Figure 1 shows the normalised density spectrum of the UCLA data. It has the general red noise (spectrum) feature (that is, most of the energy is at low frequencies) which is typical of geophysical phenomena.

This spectrum shows peaks around 28.4, 22.3 and 16.0 d. The first peak whose period is comparable to the 27 d of Rosenberg and Coleman is the most pronounced. The question, however, is whether or not this peak is statistically significant.

It is generally assumed that each of the spectral estimates has a χ^2 distribution with an equivalent number of degrees of freedom (e.d.f.). The e.d.f. is a function of the total length of the record N and the maximum number of lags (or, equivalently, of half the width of the window of the power spectrum).

Having determined the e.d.f. the significance of any of the peaks depends on whether or not persistence in the data is taken into account. This is done by comparing the density of the peak multiplied by the e.d.f. to the density of the red noise (spectrum) at that particular frequency, rather than to the density of the Gaussian white spectrum⁵.

Red noise (spectrum) is the spectrum of the persistence-modelling random

process referred to as the first order Markovian (or autoregressive) stationary process.

On the basis of our analysis all three peaks were not significant relative to the red noise (spectrum) even at the 80% confidence level which Rosenberg and Coleman arbitrarily chose. The confidence level for the 28.4-d peak is 1%. The confidence levels for the 22.3-d peak and the 16-d peak are 15% and 75% respectively.

We are therefore led to believe that the spectral peak around 28.4 d is a spurious one. Its statistical significance hinges on whether or not the reference spectrum, with which it is compared, takes into account persistence in the rainfall data.

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¹ Rosenberg, R. L., and Coleman, P. J., Jr, *Nature*, **250**, 481–484 (1974).

² Blackman, R. B., and Tukey, J. W., *The measurement of Power Spectra*, 22 (Dover, New York, 1958).

³ El-Sayed, H., and Landsberg, H. E., *Spectral analysis of the rainfall in Dakar (Senegal)*, Pub. No. 92, *The Graduate Program in Meteorology*, University of Maryland (1973).

⁴ Cooley, J. W., Lewis, P. A. W., and Welch, P. D., *The Fast Fourier Transform Algorithm and its application*, IBM Research Center, RC. 1743, (New York, 1967).

⁵ Gilman, D. L., Fuglister, F. J., and Mitchell, J. M., *J. Atmos. Sci.*, **20**, 182–184 (1963).

ROSENBERG REPLIES—El-Sayed has analysed a portion of the daily rainfall spectrum shown in his Fig. 1 by compari-

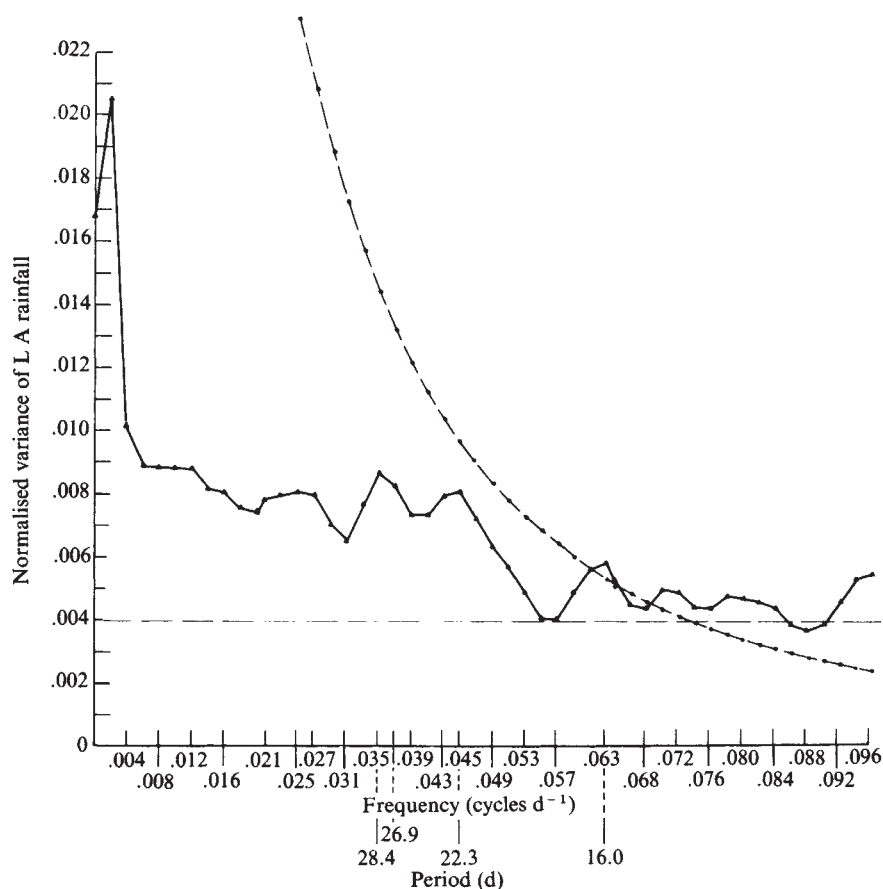


Fig. 1 The normalised variance of Los Angeles rainfall (the area under the curve is unity). The data window used is

$$W(j) = 1 - \left(\frac{j - (L-1)/2}{(L+1)/2} \right)^2; j = 0, 1, \dots, L-1$$

which gives a sharp spectral window very close to the shape of the spectral window of a cosine arch⁴, and results in an e.d.f. $\approx 3.25N/L = 83$ (ref. 3), where L is the length of the data segment for which the sample spectra are computed. The horizontal line (---) represents the white spectrum, while the curve (---) is for a red spectrum with decay coefficient (p) = 0.89.

son with a 'red noise' spectrum. In the frequency range of interest, however, the plotted red noise Markovian curve does not fit the data. He has stated (private communication) that he used a least squares fit to the pre-whitened rainfall spectrum over frequencies up to 0.5 c d^{-1} . Such a fit (which determines ρ) is overwhelmingly weighted by the high frequency power from 0.1 to 0.5 c d^{-1} , not shown in Fig. 1. To be valid, the red noise reference spectrum should fit the data equally well in the frequency interval whose peaks are being questioned and in the rest of the spectrum. For example, in the article by Gilman *et al.*² the Markovian curve used to approximate rainfall data fits all frequency intervals equally well.

El-Sayed (private communication) claims that the pre-whitening he used eliminated the annual peak. Pre-whitening (flattening the average slope of the spectrum) and recolouring should result in a spectrum with peaks (including the annual peak) whose height above the continuum is about the same as in the un-pre-whitened spectrum, but with a reduction of power leakage into the continuum. No sharp band pass filter was used to eliminate the annual peak because it is clearly evident at $1/365 = 0.0027 \text{ c d}^{-1}$ in Fig. 1. The peak-like shape and the very steep slope on its right side, which levels out immediately, indicates that the value plotted at 0.002 c d^{-1} is not part of a continuum. Figure 1 (ref. 1), leads to the spurious conclusion that the annual peak (see Table 2 of ref. 3) is not statistically significant with respect to the red noise test. If some process applied by El-Sayed reduced the height of the annual peak, which otherwise would have been above the Markovian curve, then it may have reduced the height of the ≈ 27 -d peak as well. A good Markovian fit to the 0.0 – 0.1 c d^{-1} interval could have been made by choosing the same number of points to be fit in this interval as in the 0.1 – 0.5 c d^{-1} interval. The one point at the known annual periodicity could have been excluded from this fit. In short, we believe El-Sayed applied the red noise test incorrectly. He also failed to take the cube root of the daily rainfall (before doing a spectrum) which is considered absolutely necessary to obtain a meaningful spectrum⁴ as it reduces the variance associated with anomalously large events.

If a spectral peak is not significant with respect to red noise, the conclusion is that this peak should "be relegated to the category of probable accidents that might not recur in an independent data sample"². Yet the 27-d peak occurs in each of the 5 spectra shown in Figure 2 of ref. 3. These spectra include the totally time-independent spectra for Los Angeles for 1900–35 and for 1936–71, each with 105 degrees of freedom, and spectra (each with 129 degrees of freedom) for 1927–71 for the cities of Los Angeles,

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Santa Barbara, and San Diego. The latter two cities are 300 km apart. The occurrence of the 27-d spectral peak in long, time-independent intervals is one of the most significant tests of its reality, and supports our criticism of El-Sayed's analysis.

In our study we searched for a possible correlation between rainfall and the new moon or full moon cycle (result negative)

but no other lunar cycle was examined. Using data covering 20 yr, Bryson⁵ found a statistically significant correlation between the latitude of the surface, semi-permanent, East Pacific high pressure cell and the meridional lunar force over the 27.3-d lunar cycle in declination. During this cycle, the moon moves north in declination for 13.65 d and then south for 13.65 d and, as Bryson found, pulls the Pacific high cell along with it. Lamb⁶ examined this result and stated that "the association appeared to be statistically significant". This high pressure cell acts in turn as a classical blocking high, blocking storms from reaching Southern California as in Fig. 1 of our study³.

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¹ El-Sayed, H., *Nature*, **258**, 457 (1975).

² Gilman, D. L., Fuglister, F. J., and Mitchell, J. M., *J. Atmos. Sci.*, **20**, 182–184 (1963).

³ Rosenberg, R. L., and Coleman, P. J., Jr., *Nature*, **250**, 481–484 (1974).

⁴ Stidd, C. K., *Trans. Am. geophys. Un.*, **34**, 31–35 (1953).

⁵ Bryson, R. A., *Trans. Am. geophys. Un.*, **29**, 473–475 (1948).

⁶ Lamb, H. H., *Climate: Present, Past and Future, Vol. 1, Fundamentals and Climate Now*, 219 (Methuen, London, 1972).

Cysteine and survival of transplanted thymoma

It has been shown¹ that 0.25 M cysteine could completely inhibit the growth of a transplanted thymoma, an effect claimed to be due to the ability of cysteine to inhibit collagenase.

Cysteine is usually purchased as the hydrochloride and 0.25-M solutions are strongly acidic ($\text{pH} \sim 1.0$), and, not surprisingly, cause necrosis at the site of subcutaneous injection. In the paper by Campbell *et al.*¹ it was not made clear whether the cysteine was neutral-

effect observed is thus due, almost entirely, to the injection of an acidic solution rather than to any special property of cysteine.

It should also be noted that cysteine hydrochloride given intraperitoneally to mice as an unneutralised solution is between five and ten times more lethal than neutralised cysteine.

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¹ Campbell, N. R., Reade, P. C., and Radden, B. G., *Nature*, **251**, 158 (1974).

Table 1 Effect of cysteine and cysteine hydrochloride on the growth of the transplanted PC6 plasma cell tumour

Treatment	Tumour weight (g)	Inhibition (%)
Controls	9.8, 8.1, 8.4, 9.0, 8.6	
0.25 M Cysteine*	5.6, 5.5, 5.6, 7.7, 5.3	33
0.25 M Cysteine hydrochloride	0.3, 1.6, 0, 0.6, 0	94

*The solution of cysteine was brought to $\text{pH} 7.5$ by titration with normal NaOH.

The plasma cell tumour was transplanted subcutaneously as a tumour fragment by trocar. Treatment commenced 3 d after transplantation. Samples (0.2 ml) of the appropriate solutions were injected subcutaneously as close to the tumour site as possible for five consecutive days. Animals were killed 35 d after tumour transplantation and the weight of the treated tumours compared with controls.

ised before injection and since isotonic saline was used as a control solvent, it is probable that the cysteine was not, in fact, neutralised.

Table 1 shows that a solution of cysteine hydrochloride can readily prevent the growth of a transplanted plasma cell tumour, whereas neutralised cysteine has only a very slight inhibitory action. In the experiment described in Table 1 the antitumour

CAMPBELL ET AL. REPLY—Whereas we did not attempt to buffer our cysteine solutions and acknowledge the possibility raised by Connors and Jones¹, the peak of activity that we consistently obtained at 0.25 M cysteine suggests that a specific effect is also involved.

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¹ Connors, T. A., and Jones, M., *Nature*, **258**, 458 (1975).

reviews

EXCITABLE tissue, that is, nerve and muscle, should not be tampered with lightly. True, there are all sorts of reasonable humanitarian incentives such as the existence of schizophrenia or heart failure but there is the lurking danger that the cells will take over and become the passion and obsession of the investigator. Generally a physiologist and often inadequately trained in physics and mathematics, he seems from then onwards doomed to a life of frequently frustrating self-education in those disciplines. He should find this book very helpful.

The first half is devoted to applications of linear cable theory. It is an unfortunate fact that even the simplest model of a nerve fibre (which does not even attempt to stimulate the biologically important process of lossless transmission) leads to a partial differential equation needing operational or transform methods for its solution. To follow this part of the book the reader must reconcile himself to the need to be familiar with the Laplace transform method. This done, however, he will find a very clear account of the classical study of nerve by Hodgkin and Rushton, originally published in 1946, its application to the analysis of endplate potentials by Fatt and Katz and its elaboration by Rall to synaptic potentials in central neurones regarded as

Lots of potential

Electric Current Flow in Excitable Cells. By J. B. Jack, D. Noble and R. W. Tsien. Pp. xvi+502. (Clarendon: Oxford, Oxford University: London, August 1975.) £18.00.

geometrically non-uniform cables. The authors' intention was evidently not to produce a neat compendium of formulae for immediate numerical exploitation. On the contrary, there is a certain deliberate and, I think, attractive untidiness about the treatment, some results being stated without proof, others being derived in several different ways with different degrees of completeness; some points are dwelt on because of their mathematical, and others because of their physiological interest.

The second half of the book is concerned with action potentials. Dealt with in turn are the non-linear electrical properties of membranes—which are, of course, what make action potentials possible—the excitation process, conduction and repetitive activity. As is well known, non-linear differential

equations do not in general succumb to analytical methods, but as the authors show in their last chapter, such methods can produce significant information if the function representing the electrical behaviour of the membrane is chosen with sufficient ingenuity. On a first reading, this half of the book was somewhat less satisfactory. Parts of the chapter on non-linear membrane properties seem unnecessarily difficult to follow and the reader is too frequently directed to external references for points of substance. In the chapter on repetitive activity, the theoretical discussion sometimes seems too weighty for the experimental data. These are minor criticisms, however, in the light of the enormous amount of material presented.

The book is extremely well written at a detailed level. It is also well produced: among the very few mathematical misprints I noticed, only one, the omission of a pair of brackets from equation 3.60, was catastrophic. In their introduction the authors write that they were primarily motivated "... by the desire to make what is admittedly one of the most difficult areas of physiological theory less forbidding and more accessible ...". I think that they have succeeded.

B. L. Ginsborg

THIS book presents to a wider audience the papers prepared for a small international symposium held in March of this year. There are three introductory papers on the immune response, auto-immune phenomena and side effects of immunisation by insulin as a model system. All will be of interest to endocrinologists whose knowledge of immunology requires updating. Eight papers are grouped under the heading "Basic Research", and vary considerably in scope, content and value, ranging from quite lengthy reviews of original work and the published literature through excellent short reports of well-defined, although limited, projects to scrappy accounts of rather diffuse studies, so incomplete in performance or reporting (or both) as to be of little value. The four concluding papers deal with attempted or projected practical applications and here the high standard set by the introductory papers is maintained. Topics discussed are: immunisation with a steroid hormone-protein conjugate to control the

Immunological use for hormones

Immunisation with Hormones in Reproduction Research. (Proceedings of the International Symposium.) Edited by E. Nieschlag. Pp. x+253. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl.55; \$22.95.

development of boar taint in maturing intact male pigs; immunisation with gonadotrophins to control reproduction in dogs; the prospects for and early experiments on the control of human fertility by immunisation with the protein hormone produced by the early human conceptus chorionic gonadotrophin; and a brief account of the use of homologous antibodies raised in a primate against pituitary luteinising hormone from a subprimate to reversibly control the development of the corpus luteum in sterile cycles and to

terminate pregnancy in fertile cycles in other members of the same primate species.

The papers were clearly written by enthusiasts yet surprisingly the common feature of many of them is the emphasis on the difficulties and uncertainties of both the practical procedures and of the interpretation of the results obtained. This impression might well have been stronger had the discussions after each paper been reported in full rather than in the form of a quite unrewarding and frustrating synopsis. Nonetheless this is a useful book for any biologist who wants a recent account of work in an area which is still of great interest even though it seems to be rather less promising, in terms of either basic research or practical application, than it was hoped it would be. The production of the book, from the remarkably error-free typescripts supplied by the participants, is excellent and has been commendably prompt.

K. Brown-Grant

Gas-phase microwave spectroscopy

Molecular Rotation Spectra. By H. W. Kroto. Pp. xi+311. (Wiley-Interscience: London and New York, March 1975.)

THERE are at least seven books on gas-phase microwave spectroscopy. The subject provides many very satisfying examples of successful application of quantum mechanics to models which are simple (for example the rigid rotor) yet fit experimental microwave data in a convincing manner. Furthermore, these theoretical treatments have made possible the determination of reliable structures, dipole moments, field gradients, barriers to internal rotation, molecular quadrupole moments, force constants, conformations, and so on, for a large number of molecules. The classic book, in this reviewer's opinion, remains that of Townes and Schawlow (*Microwave Spectroscopy*) which is, however, now twenty years old. A more recent work of extensive coverage is that of Gordy and Cook (*Technique of Organic Chemistry*, 1972). Any additional book has to compete with a number of very excellent volumes.

Kroto covers most of the usual areas: energy levels of the rigid rotor, selection rules and intensities, Stark effect, nuclear quadrupole interactions, internal rotation, vibration-rotation

interaction, linear molecules with electronic angular momentum, and, rather briefly, experimental methods.

There are reports of recent work not contained in earlier books—notably the contributions of Watson to the molecular Hamiltonian and Longuet-Higgins to the symmetry classification. Further, the general approach is somewhat different. Instead of complete coverage of the many possible topics with references to the literature for derivations, Kroto has omitted a number of effects (for example, the Zeeman effect of diamagnetic molecules) but has attempted to present more complete derivations. It is a highly personal judgement as to whether he has succeeded in clarifying all these derivations; certainly for many, more elementary treatments are available. On the other hand, the book provides excellent examples of more advanced methods. The first chapter is mainly devoted to quantum mechanical principles but it seems doubtful that it would be too helpful to someone who had not already mastered the basic ideas.

This book has more than the usual space devoted to some of the practical methods of analysing spectra and has many figures of well chosen spectra to illustrate various points.

Altogether, this new work is a welcome addition to the field but is not a substitute for the larger books with more complete coverage, more tables, larger bibliographies, and so on.

E. Bright Wilson

Alkaloid miscellany

The Alkaloids: Chemistry and Physiology, Volume 15. Edited by R. H. F. Manske. Pp. xv+315. (Academic: New York and London, July 1975.) \$39.50; £19.75.

THIS latest review volume maintains the style of previous issues with contributions on the ergot alkaloids by P. A. Stadler and P. Stütz and on the Amaryllidaceae alkaloids by C. Fuganti, subjects previously reviewed in volumes 8 (1965) and 11 (1968) respectively. Two chapters consider topics not covered by specific reviews in previous issues. S. Yamamura and Y. Hirata have provided a first review for the series on the *Daphniphyllum* alkaloids, compounds containing the 2-azabicyclo [3.3.1] nonane system isolated from the Japanese tree, *Daphniphyllum macropodum*, and related species, whereas R. Tschesche and E. U. Kaussmann have compiled a chapter on the cyclopeptide alkaloids, substances on which research has focused within the past decade. This account is primarily chemical and includes a major section on spectral methods, largely mass spectrometry, whereas present biochemical and pharmacological knowledge merits only a page. In contrast, pharmacology and toxicology are the centres of attention in a section by V. Preininger on the Papaveraceae alkaloids other than morphine and codeine, chemical aspects having been reviewed recently elsewhere. R. H. F. Manske ends the book with his regular sifting of the journals entitled "Alkaloids unclassified and of unknown structure" (no reference after 1973), including four entries referencing alkaloids from Coleoptera.

This volume will find its main market in the specialist libraries as a most valuable reference work. Its formidable price and the detail of the presentation will deter all who do not possess a special interest in the alkaloids. The active researcher in the field, having a good grasp of the primary research literature in his particular area, will probably be most immediately interested in the bibliographies which, however, frequently and necessarily include much which is not recent. Thus, in the chapter on ergot alkaloids, the median date of references is 1968.

The present volume, which admirably maintains the high standards of the series, will constitute necessary reading for scientists of different disciplines who share a common interest in the alkaloids, and should become an important addition to the shelves of many libraries.

E. S. Albone

Catalysis, great and small

Catalysis in Micellar and Macromolecular Systems. By Janos H. Fendler and Eleanor J. Fendler. Pp. xii+545. (Academic: New York and London, July 1975.) \$44.00; £21.10.

SINCE the pioneering work of Duynstee and Grunwald was published in 1954 there has been a growing interest in the catalysis of organic reactions by micelles. Much of this interest has been generated by the hope that micelles might mimic enzymes in their catalytic powers. Unfortunately, although the Michaelis-Menten mechanism is useful for the description of the kinetics of micelle-catalysed reactions, micellar catalysis, in aqueous solution at least, has neither the efficiency nor the specificity of enzyme catalysis. Nonetheless, recent work on reversed micelles (that is, those enclosing hydrophilic regions of molecular dimensions in a pre-

dominantly hydrophobic environment) suggests that catalysis in reversed micelle systems will more closely resemble that by enzymes.

The authors of this book have contributed a great deal to our knowledge of this topic, and have produced what must surely be the definitive volume for some time to come. The title is perhaps misleadingly restrictive, as the authors have included much informative background material on the structure of micelles and the thermodynamics and kinetics of their formation. A noteworthy feature of the book is the lengthy tables which collate all published information on micellar catalysis up to June 1974. A supplement at the end of the book updates the literature survey to December 1974. Both the authors and the publishers are to be commended on the short time interval between the most recent reference and the publication of the book. This book will be an essential purchase for the library of all laboratories concerned with physicochemical studies on surfactants.

J. E. Crooks

Muscle receptors

Muscle Receptors. (Handbook of Sensory Physiology, Volume 3, Part 2.) Edited by C. C. Hunt. Pp. viii+312. (Springer: Berlin and New York, 1974.) DM104; \$42.50.

THIS volume is made up of three different sections, each by a different author. A long (190 pages) section by Barker on the anatomy of muscle receptors is followed by much shorter sections on their physiology (Hunt) and central actions (McIntyre).

The anatomical section is a very full review of the present state of the subject with numerous illustrations and a comprehensive bibliography; it fills a real need and it will serve as a valuable reference book. The greater part of this section deals with muscle spindles, although other receptors are also very adequately treated. Many aspects of muscle spindle anatomy and its relationship to function remain controversial—and here Barker's own views come across quite clearly—although he takes care to mention the points of disagreement.

In the other two sections of this volume the authors have been in some difficulty: Matthews' very full account

of the properties of mammalian muscle receptors appeared only two years earlier, and there was clearly no need for another comprehensive review.

Hunt deals briefly with many of the properties of muscle receptors and then gives a more detailed account of some recent work on muscle spindles, and especially on the relationship between the different fusimotor effects, the different types of motor ending and the activity of different intra-fusal fibres. There is also useful information about some non-mammalian species.

McIntyre has written a lucid account of the central actions of impulses from muscle afferents, starting with a useful section on methods of selective activation of afferent fibres. By treating each part of the subject in a historical way he builds up a clear picture of the present position and the present problems. This section is brief and somewhat limited in scope, but within these limitations it is excellent.

Hunt gives, therefore, useful information on physiological work of the last year or two and McIntyre provides a section that serves as a valuable introduction to the central actions of muscle afferents; but it is the long section by Barker on anatomy that will make many teachers, research workers and advanced students wish to have this book on their shelves.

Peter M. H. Rack

Fundamentals of neurophysiology

Fundamentals of Neurophysiology. Edited by Robert F. Schmidt. Pp. ix+293. (Springer: Heidelberg, Berlin and New York, 1975.) DM29; \$12.50.

THE cost of most reference physiological textbooks has created a demand for less expensive paperback versions. Unfortunately for those who teach neurophysiology these condensed paperback versions usually give an inadequate account of that subject. *Fundamentals of Neurophysiology* is a paperback English edition of a German textbook based on a programmed textbook of a series of lectures to first-year physiology students.

The book is divided into eight sections. Those concerned with excitation of nerve and muscle, synaptic transmission and properties of muscle are very good. The sensory system is well presented although there is an inadequate account of the physiology of pain. The sections concerned with reflexes, the motor system and the autonomic nervous system are based on the hypothesis that there is a hierarchical organisation within the nervous system with "higher" and "lower" centres. The higher centres control the lower, and together they somehow mould a great variety of stereotyped reflexes into what we know as movement and posture. This was a very productive hypothesis in the last century but it has been falsified so often in this century that its use in a modern textbook of neurophysiology is questionable.

After each major topic within a section are a series of questions which the reader is advised to answer before proceeding. This method of self assessment, if it is to be successful, requires careful attention to the content and phrasing of both questions and answers. Unhappily in some sections both the questions and some choices given for answers are trivial.

Apart from that and minor points of disagreement which are undoubtedly the result of having to simplify and condense controversial concepts, the authors have written an excellent textbook for most neurophysiological courses. The text is well written, the illustrations are unambiguous, and a consistent, adequate standard for the medical physiology student is maintained. Unfortunately the price will put this book beyond the reach of all but the most affluent student.

T. H. Koeze

Combating drought

Physiological Aspects of Dryland Farming. Edited by U. S. Gupta. (Oxford and IBH: New Delhi, Bombay and Calcutta, 1975.) RS.80.00.

DROUGHT is the major factor limiting world food production and its occurrence is inevitable, but its effects may be reduced by developing a dry farming technology based on an understanding of plant-water relationships. Eleven different authors review aspects of this subject, beginning with a detailed consideration of the physiological principles of dryland crop production by Arnon (Israel). There follow reviews of drought injury and resistance of crop plants by Larson (US), root patterns in crop plants by Hurd and Spratt (Canada), the role of mulches by Unger (U.S.A.) and the effects of humidity on crop growth by O'Leary (U.S.A.) and of wind by Sturrock (New Zealand).

As almost all the water taken up by the roots is transpired to the

atmosphere by the leaves there is considerable interest in the use of anti-transpirants to reduce water losses from the leaves and this aspect is discussed by Davenport and Hagan (US). As photosynthesis proceeds much dry matter is lost through photorespiration and the biochemistry of this process is described by Goldsworthy (UK), who also speculates on the remote possibility of reducing this loss in the future—either by using chemicals or by the development by plant breeders of a wider range of crops with C-4 metabolism which do not seem to photorespire. Finally, Iwata (Japan) summarises information on the heat unit concept of crop maturity and discusses its application in crop production. Each chapter is supported by a long list of references to published literature for those wishing to probe deeper.

Although the emphasis is on dryland farming the principles discussed apply also to crops grown in more favourable water regimes, and this book has a much wider value to agronomists, crop physiologists, plant physiologists, botanists and ecologists than that suggested by the title.

J. D. Ivins

To flower or not to flower . . .

Photoperiodism in Plants. (European Plant Biology Series.) By Daphne Vince-Prue. Pp. xiv+444. (McGraw-Hill: London and New York, May 1975.) £9.95.

PHOTOPERIODISM is one of the most exciting, and yet one of the most mystifying, aspects of the regulation of plant development. It is an extremely difficult topic to teach, and consequently often receives scant attention in university courses. That is unfortunate, since it offers an almost unique opportunity to study the mechanisms of developmental control. The induction of flowering involves a change from the steady-state, non-determinate formation of vegetative organs at the shoot apex, to a completely new, determinate, sequence of events in which a series of organs, never previously produced by the plant, are formed. Those organs—which together form the flower or inflorescence—although having morphological homologies with the vegetative organ are anatomically and structurally quite different and often produce metabolites and secondary products not synthesised under vegetative conditions. This major redirection of development, inducible merely by a subtle change in the daily length of the dark period, must surely involve, as a causal step, the selective and qualitative regulation of the expression of genetic information. In no other organisms can the whole pattern of organ development be so readily manipulated, and one might justifiably hope that investigations into photoperiodism in higher plants would yield information and concepts of central importance to our understanding of development.

That this happy outcome has not yet materialised, is probably largely attributable to the diffuse nature of our current knowledge of photoperiodism. Many potential investigators must be discouraged by the need to cope with so much apparently unrelated information. What Dr Vince-Prue has attempted to do in this book is to assist the reader to see the wood through the trees. The easy way to do that, of course, is for the author to ignore the great diversity of information, and merely present a personally selected, unified view. Dr. Vince-Prue has eschewed that approach, and throughout the book has unflinchingly provided the reader with the available information, presented in an admirably clear, concise, and, above all, readable form; where logical conclusions can be drawn, she draws them, but she is not

unwilling to leave unresolved questions open. In my opinion, that is exactly the right approach, and Dr Vince-Prue succeeds in her formidable enterprise through her immense knowledge and exceptional understanding of the topic.

The book is logically organised and covers all those aspects necessary for a sound understanding. It will prove to be an excellent book for university teachers and research workers, and many undergraduates will find it invaluable as a source-book. Particularly useful are several chapters on the photoperiodic control of development other than flowering.

After reading the book with enjoyment, however, I still find myself impressed more by the diversity of the information available, than by the power of the unified concepts derived from that information. This is a reflection of the subject though, not of the book, and it may be why Dr Vince-Prue has quoted in her Preface a beautifully enigmatic line from Hamlet: "Why day is day, night night, and time is time."

Harry Smith

More than black and white

Race Differences in Intelligence. (A Series of Books in Psychology.) John C. Loehlin, Gardner Lindzey and J. N. Spuhler. Pp. xii+ 380. (Freeman: San Francisco and Reading, June 1975.) £3.00.

THE goal of this book is to consider the available evidence on the relative importance of genetic and environmental variation in accounting for racial-ethnic IQ differences in the United States. The book is divided into three sections. The first considers the background and recent history of the race-IQ controversy and deals with three of the key concepts on which the controversy focuses: the concepts of race, intelligence and heritability. The second section deals with the empirical evidence, and considers a wide number of studies. The final section contains the conclusions and also the authors' views on the implications of these conclusions for the formulation of social policy.

The value of this book is that it provides an excellent summary of the available data on studies dealing with the race-IQ question. But my initial enthusiasm for the book gradually declined as I realised that the authors have skimmed over some of the most important issues. Their discussion of the controversy is far from complete.

The authors consider some of the problems associated with cultural bias

in IQ tests and point out that it may be impossible to identify test items "culturally fair" to a particular group. But then they side-step the question of whether the existence of cultural bias in tests makes all attempts at cross-cultural comparison meaningless. But it is when discussing heritability that this book really falls down, as in fact do all arguments concerning race-IQ differences. The authors do not seem to have grasped that fact.

They still subscribe to the belief that a knowledge of the heritability of a trait within a population can tell us something about between-group differences. That is just not so. A measure of heritability is only relevant within the population and environment in which it was measured. No comparison between populations can be made from a heritability measure. It is well known that American blacks are far worse off than whites for a number of environmental factors known to influence performance on IQ tests substantially. These include educational inferiority, lower nutritional levels, cultural biasing in tests, racial prejudice, lack of incentive, to name but a few; as well as the fact of being black in a white dominated society. We have no way of correcting test scores for these systematic differences to obtain unbiased figures. It is impossible to make *a priori* judgements of different aspects of the environment, as every ecologist and plant physiologist knows.

The authors believe that a heritability measure is of use in the present controversy since we are discussing the effect of the modification of environmental variables which are within the existing range of environments. This assumes an additivity of effects, for which there is no evidence. Large differences in traits which are highly heritable can be caused solely by environmental differences. Environmental changes such as the amount of trace elements in the soil can have profound effects on genotypic expression. On the other hand, even if an observed difference is genetic it does not mean that a trait cannot be altered substantially by an appropriate environmental change. An example is the treatment of phenylketonurics with a diet deficient in phenylketonuria. It is meaningless to try and separate genetic and environmental contributions to IQ differences between racial groups.

The authors conclude that some research on differences in ability between US racial-ethnic groups seems to be of fairly high scientific and social priority. It is difficult to see how they can reach that conclusion. From their extensive discussions on the studies quoted the authors have demonstrated that the whole race-IQ question is in fact unanswerable.

Glenys Thomson

Animal mechanics

How Mammals Run: Anatomical Adaptations. By P. P. Gambaryan. Pp. xiv+367. (Israel Program for Scientific Translations: Jerusalem; Wiley: New York and Chichester, April 1975.) £10.80.

GAMBARYAN and his colleagues have assembled a large body of knowledge on terrestrial locomotion which has hitherto been available only in Russian, and little known in the West. They have dissected, and measured the limb bones and muscles of, about a hundred species from seven orders of mammals and filmed nearly half these species running at various speeds. Their data was collected, with data from other sources, and translated into English.

The book includes a large collection of illustrations traced from film sequences and still photographs. There are a great many illustrations of small mammals, as well as of the larger ones which are well illustrated in other books such as Muybridge's *Animals in Motion*. These illustrations are well chosen, and Gambaryan makes good use of them. His comparison of the gallop and ricochet and discussion of the circumstances in which each evolved is particularly interesting.

The data on muscle weights is extensive, but of limited use. Gambaryan is well aware of the significance of moment arms, cross-sectional areas and pennation patterns, but tells us little about them.

Recent Western research on terrestrial locomotion has used a wide variety of techniques including light and X-ray cinematography, electromyography, force transduction and measurement of oxygen consumption. The Russian work reported in this book used a more restricted range of techniques. No X-ray cinematographs or measurements of oxygen consumption were made. Electromyography was carried out but only on the cat, duplicating work done in the West. Force platforms were used but they were capable of registering only vertical components of force.

Much of the book is devoted to comparisons between mammals, some of which are most enlightening but others are unconvincing because of lack of rigorous mechanical analysis. Assumptions are made about the directions of the forces exerted by feet which conflict with all force platform records, including those in Manter's paper of 1938 (which is cited). The attempts at theoretical mechanical analysis are more ele-

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One of Muybridge's illustrations

mentary and less penetrating than the analysis in Smith and Savage's paper of 1956 (which is cited). There is no appreciation of the roles of elastic storage of energy or of negative work in running.

This is a valuable book but it is not an adequate account of current knowledge in this subject.

R. McNeill Alexander

Structural Materials in Animals. By C. H. Brown. Pp. xv+448. (Pitman: London, July, 1975.) £10.50.

STRUCTURAL studies on the fibrous molecules of skeletal systems in organisms were important contributions to the early growth of Molecular Biology. Nowadays it is more fashionable to identify Molecular Biology with the study of enzymes and nucleic acids, and the problems involved in synthetic, metabolic and genetic processes. Happily, the fibrous molecules have not been entirely neglected. Several excellent books on the chemistry and conformation of structural biopolymers have appeared recently. Dr Brown's addition to this literature is a thorough, somewhat laborious, account of how and where these materials occur throughout the animal kingdom. Her book is aimed primarily at zoologists, who will be familiar with the standard textbook approach.

An elementary introduction to the chemistry, conformation and molecular organisation of the main structural molecules prefaces detailed descriptions of their occurrence and incorporation into skeletal structures and tissues in each phylum from Protozoa to Chor-

data. A short chapter on decay of structural materials is followed by some general conclusions in which an attempt is made to develop a more comparative theme. The style of writing is clear, direct and unpretentious, but in common with many similar catalogues of research results, the narrative is apt to be pedestrian. In general the illustrations are well chosen for their good quality and pertinence to the text, but some diagrams seem to be superfluous. For example, the diagram showing the comparative volume of coelenterates occupied by mesogloea may have done much to clarify the original article from which it has been taken, but in this book it adds little to the accompanying text.

There are a number of trivial factual mistakes, and the text is littered throughout with spelling and printing errors—a deplorable feature which is all too common in scientific volumes nowadays. But the worst feature of this book is the inexcusable and inordinate number of bibliographical errors. So many authors' names are mis-spelled in the text or reference list, and sometimes in both. One particular name is spelled in three different ways in the space of two pages, whereas some others appear in two different versions on the same page. Inattentive proof reading—or none at all—has devalued what is otherwise a useful book. Such errors may not deter the general reader, but for research workers who may wish to consult this book these mistakes will be a constant source of irritation. Furthermore, it is very frustrating when (on at least one occasion) a paper quoted in the text is not listed in the references.

W. Kenchington

obituary

Sir James Wilfred Cook, FRS, sometime Regius Professor of Chemistry in the University of Glasgow and Vice-Chancellor of the Universities of Exeter and East Africa, died on October 21 at the age of 74.

After graduating from University College, London, Sir James became lecturer in chemistry at the Sir John Cass Technical Institute in 1920, where he obtained his PhD on the chemistry of anthracene derivatives. Sir Ernest Kennaway invited him to join the staff of the Royal Cancer Hospital in 1929, where he remained for ten years, becoming reader in pathological chemistry in 1932 and professor of chemistry in 1939. It was at this hospital that he made his outstanding contributions to cancer research, by demonstrating the carcinogenicity of polycyclic benzenoid hydrocarbons and then isolating from coal tar its main carcinogenic component. This work showed for the first time that cancer could be induced by minute quantities of a pure chemical compound. Further work revealed a correlation between structure and carcinogenicity. Cook and Kennaway were awarded the Prize of the International Union against Cancer, and Cook was elected a fellow of the Royal Society in 1938, in recognition for their work. In 1939 he was appointed Regius Professor of Chemistry at Glasgow, a chair which he occupied for sixteen years. While coping ably with his new

administrative duties he also found time to act as President of the Royal Institute of Chemistry from 1949–51 and as a member of the University Grants Committee from 1950–54. He continued to work on polycyclic hydrocarbons, and also carried out research in colchicine and the tropolones. In 1954 he became Principal of the University College of the South West and negotiated its conversion to the University of Exeter, becoming its first vice-chancellor in 1955. He was instrumental in bringing about the emergence of a full-blooded university. His honorary directorship of the MRC Carcinogenic Substances Research Unit, at Exeter, helped to keep him in touch with organic chemistry. Cook had always shown an interest in education in developing countries, and on retiring in 1965, became vice-chancellor of the University of East Africa. In 1974 he became Chairman of the Academic Advisory Committee of the New University of Ulster, at Coleraine, and again displayed his enthusiasm in the cohesion of a new venture. He was presented with a knighthood in 1963 and was a Davey Medallist of the Royal Society (1954).

Gustav Hertz, the German Nobel Prize winning physicist, died in East Berlin on October 30, at age of 88.

Dr Hertz, who was a nephew of Heinrich Hertz (renowned for his work

on electromagnetic waves) was educated at the Universities of Gottingen, Munich and Berlin. In 1925, he became Professor of Physics at the University of Halle, and in 1928, Director of Physics at the Berlin-Charlottenberg Technische-Hochschule. In 1934, he was obliged to resign from his academic post, since he was unwilling to take the oath of allegiance to the newly-established Nazi regime. From then on, and until the end of the war, he worked for the Siemens company, who built a special laboratory for him. At the end of the war, he went to the Soviet Union, where until 1954 he worked on the Soviet atomic bomb project. He then returned to East Germany, and taught physics at the University of Dresden, until his retirement in 1961. Hertz's Nobel Prize, which he won with Dr J. Franck in 1925, was for work on the ionisation theory of gases, which played a fundamental role in the establishing of quantum theory. During this similar period of his research activity, he also devised a diffusion method of calibrating neon isotopes. His role in the development of the Soviet atomic bomb is difficult to estimate, not only from the classified nature of the project, but also because, as Sakharov has explained, the situation was a complex one of "collective invention". The fact that Hertz was given the Lenin Prize suggests, however, that his contribution was considerable.

Vera Rich

announcements

Awards

Her Majesty the Queen has approved the recommendations made to the Council of the **Royal Society** for the three **Royal Medals** to be awarded as follows: To **E. Bullard** in recognition of his distinction as a world leader in geophysics; to **D. C. Phillips** in recognition of his solution of the three-dimensional structure of an enzyme and his contributions to the techniques of X-ray crystallography; and to **B. Wallis** in recognition of his contributions to aeronautical engineering.

Appointment

A. Alexandrov has been elected president of the Soviet Academy of Sciences.

Reports and publications

Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1395-J: The Supai Group—Subdivision and Nomenclature. By Edwin D. McKee. Pp. iii + 11. Professional Paper 437-F: Land Subsidence Due to Ground-Water Withdrawal in the Los Banos-Kettleman City Area, California, Part 2. Subsidence and Compaction of Deposits. By William B. Bull. Pp. v + 90. Professional Paper 925: Sedimentation and Tectonics in the Early Tertiary Continental Borderland of Central California. By Tor H. Nilsen and Samuel H. Clarke, Jr. Pp. iv + 64. (Washington, DC: Government Printing Office, 1975.) [710]
United States Department of the Interior: Geological Survey. Water-Supply Paper 2152: Quality of Surface Waters of the United States, 1970. Part 2: South Atlantic Slope and Eastern Gulf of Mexico Basins. Pp. x + 668. (Washington, DC: Government Printing Office, 1974.) \$5.45. [810]
Studia Forestalia Suecica. Nr. 123: Die Kiefern-rindenwanze, *Aradus cinnamomeus* Panz. (Hemiptera-Heteroptera), Ein Beitrag zur Kenntnis der Lebensweise und der Forstlichen Bedeutung. Von Leo Brammanis. Pp. 81. Nr. 124: Matning Analys och Optimering av Skogsmaskiners Driftsakerhet. (Logging-machine Failure Avoidance: Identification and Measurement of Key Parameters). By Sven-Ake Axelsson. Pp. 38. Nr. 125: Produktionen i Kulturbestand av ek i Sodra

Sveige. (Yield of Oak Plantations in Southern Sweden). By Charles Carbonnier. Pp. 89. Nr. 126: The Relationship Between Self-Fertilization, Empty Seeds and Seeds Originating from Selfing as a Consequence of Polyembryony. By Dag Lindgren. P. 34. (Stockholm: Skogshogskolan, Royal College of Forestry, 1975.) [910]

United States Department of the Interior: Geological Survey. Professional Paper 786: Pleistocene Geology of the Northeast Adirondack Region, New York. By Charles S. Denny. Pp. iv + 50 + plates 1–7. (Washington, DC: Government Printing Office, 1974.) \$1.45. [910]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2097: Quality of Surface Waters of the United States, 1968. Part 8: Western Gulf of Mexico Basins. Pp. x + 473. \$3.45. Water-Supply Paper 2118: Surface Water Supply of the United States, 1966–70. Part 6: Missouri River Basin. Vol. 3: Missouri River Basin from Sioux City, Iowa, to Nebraska City, Nebraska. Pp. xiii + 710. \$3.45. Water-Supply Paper 2120: Surface Water Supply of the United States, 1966–70. Part 7: Lower Mississippi Basin. Vol. 1: Lower Mississippi River Basin Except Arkansas River Basin. Pp. xi + 1274. Water-Supply Paper 2127: Surface Water Supply of the United States, 1966–70. Part 10: The Great Basin. Pp. xi + 1143. \$7.35. Water-Supply Paper 2145: Quality of Surface Waters of the United States, 1969. Part 6: Missouri River Basin. Pp. xi + 441. \$3.25. (Washington, DC: Government Printing Office, 1973, 1974 and 1975.) [1010]